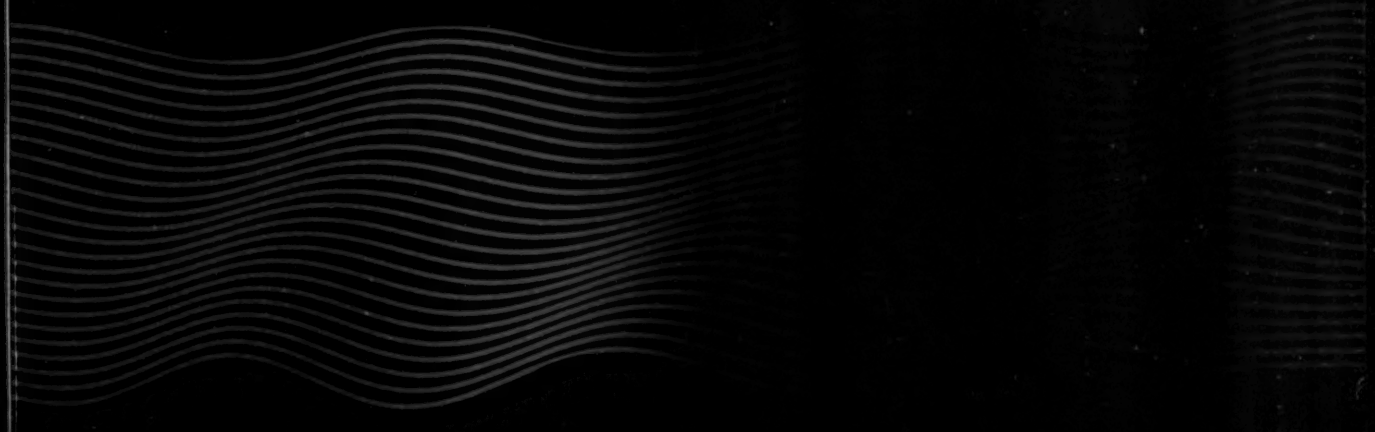
National Research Council Canada
Conseil national de recherches Canada

NRC · CNRC

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques



The *Canadian Journal of Fisheries and Aquatic Sciences* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, and *Journal of the Fisheries Research Board of Canada* 1938–79. The *Journal* is published by the National Research Council of Canada in cooperation with the Department of Fisheries and Oceans.

The *Journal* is issued monthly to form annual volumes of twelve numbers plus Supplements.

Editorial Policy

The *Journal* publishes original research articles, critical reviews, perspectives (essays of opinion or hypothesis), and comments. Papers may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and they should lead to identifiable conclusions or syntheses, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of fisheries and aquatic sciences. They should demonstrate clearly a contribution to knowledge beyond the confirmation state. Originality should relate to more than the particular (a certain year, place, taxon, or chemical compound) such that existing understanding is reformulated or extended.

It would assist the Editors if prospective authors identified briefly by covering letter (a) aspects of their papers that meet the foregoing objectives, (b) potential referees, and (c) other manuscripts contemplated or in press containing the same or similar information.

Submissions in English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. The use of proprietary names does not imply endorsement of the product or company.

Guides

A guide for authors appears in the first issue of each volume and is available free from the *Journal*.

Reprints

Reprints may be purchased at the time of publication (an order form is sent out with the galley proof). All orders should be sent back with the galley proofs. Reprint phone enquiries: Eileen Fink at (613) 993-0151.

Advertisements

Advertisement rates and information can be obtained from Hoda Jabbour at (613) 993-9085. Acceptance of an advertisement, announcement, or other material does not imply endorsement by either the *Journal* or the National Research Council of Canada.

Microfilm

Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

The publisher grants permission to individuals who wish to quote short excerpts and reproduce figures, tables, or photographs from articles in this journal, provided that the source of such material is fully acknowledged. As a courtesy the consent of authors of such material should be obtained.

©National Research Council of Canada, 1993. World rights reserved.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by the National Research Council of Canada for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of $3.00 per copy is paid directly to CCC, 21 Congress Street, Salem, MA 01970, U.S.A. 0008-4212/92 $3.00 + 0.00.

The above permission does not extend to other kinds of copying, such as copying for general distribution, for advertising, or promotional purposes, for creating new collective works, or for resale. For such copying, arrangements must be made with the publisher.

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979. Le *Journal* est publié par le Conseil national de recherches du Canada en collaboration avec le ministère des Pêches et des Océans.

Le *Journal* paraît tous les mois, formant ainsi un volume de douze numéros, et offre en plus jusqu'à deux suppléments par année.

Politique de rédaction

Le *Journal* publie des articles fondés sur une recherche originale, des critiques, des essais portant sur une opinion ou une hypothèse (perspectives) et des commentaires. Les textes peuvent avoir trait aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influent sur les systèmes de production aquatique. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. Ils doivent clairement démontrer qu'ils contribuent aux connaissances en faisant plus que corroborer des faits. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles.

Les auteurs éventuels aideraient les rédacteurs s'ils identifiaient brièvement, dans une lettre d'accompagnement a) les aspects de leurs textes qui répondent particulièrement aux objectifs indiqués ci-dessus, b) des arbitres possibles et c) d'autres manuscrits envisagés ou sous presse, dont la teneur est identique ou se rapproche de celui qui est soumis.

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le *Journal* décline toute responsabilité quant aux énoncés des contributeurs. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

Guides

Le guide des auteurs paraît dans la première livraison de chaque volume et est offert gratuitement par le *Journal*.

Tirés à part

Les tirés à part peuvent être achetés au moment de la publication. Un bon de commande à cet effet accompagne les épreuves de galée et doit être retourné avec celles-ci. Renseignements supplémentaires : Eileen Fink au (613) 993-0151.

Annonces publicitaires

Pour toute information concernant les annonces publicitaires ou leur coût, s'adresser à Hoda Jabbour au (613) 993-9085. La publication d'annonces publicitaires, d'avis et autres n'implique pas l'endossement de leur contenu par le *Journal* ou le Conseil national de recherches du Canada.

Microfilms

On peut acheter sur microfilms de 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de University Microfilms International, 300 North Zeeb Road, Ann Arbor, Michigan 48106, É.-U.

L'éditeur accorde aux particuliers la permission de citer de brefs extraits et de reproduire des figures, des tableaux ou des photos dans les articles du *Journal*, à la condition que la source soit indiquée explicitement. Dans ce cas, il convient, par courtoisi, d'obtenir le consentement des auteurs.

©Conseil national de recherches du Canada 1993. Tous droits réservés.

Le Conseil national de recherches du Canada autorise les bibliothèques et autres utilisateurs inscrits au Copyright Clearance Center (CCC) Transactional Reporting Service à photocopier les documents destinés à un usage interne ou personnel, ou à l'usage interne ou personnel de clients particuliers, à la condition que le tarif de base de 3 $ par copie soit payé directement au CCC, 21 Congress Street, Salem, MA 01970, U.S.A. 0008-4212/92 3,00 $ + 0,00.

La présente permission ne s'applique pas à d'autres genres de reproduction, notamment la reproduction en vue d'une distribution générale, à des fins de publicité ou de promotion, pour la création de nouveaux travaux collectifs, ou pour la revente. Dans ces cas, il faut prendre les dispositions qui s'imposent en communiquant avec l'éditeur.

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 50, No. 8, August 1993 **Volume 50, n° 8, août 1993**

D.G. Cook — *Editor/Rédacteur*
M.M. Milne — *Assistant to the Editor/Adjointe au rédacteur*
P.E.K. Symons — *Consulting Editor/Rédacteur-conseil (Victoria, B.C.)*
N. Moir — *Consulting Editor/Rédactrice-conseil (Nepean, Ont.)*

Editorial Board/Conseil de rédaction

L.M. Dickie — *DFO/MPO, Dartmouth (Advisor/Conseiller)*
J.D. Neilson — *CFRAMP, St. Vincent (Associate Editor/Rédacteur associé)*
L.S. Parsons — *DFO/MPO, Ottawa (Advisor/Conseiller)*
R.M. Peterman — *Simon Fraser University (Advisor/Conseiller)*
J.C. Roff — *University of Guelph (Advisor/Conseiller)*
P. Schwinghamer — *DFO/MPO, St. John's (Deputy Editor/Rédacteur adjoint)*
N.H.F. Watson — *DFO/MPO, Halifax (Associate Editor/Rédacteur associé)*

Research Journals/Revues scientifiques

Bruce P. Dancik — *Editor-in-chief/Directeur général*
Iain E. P. Taylor — *Assistant Editor-in-chief/Directeur général adjoint*

Publishing Department/Service de publication

Aldyth Holmes — *Director/Directrice*
Joan E. Hill — *Operations Manager/Responsable des opérations*
Christine Shugar — *Head of Publications/Chef des publications*
Peter M. Burke — *Publication Officer/Agent de publication*

Editorial Office:

Research Journals
National Research Council of Canada
Ottawa, Ontario, Canada
K1A 0R6
Telephone (613) 993-2209
FAX (613) 952-7656

Bureau de rédaction :

Revues scientifiques
Conseil national de recherches du Canada
Ottawa (Ontario), Canada
K1A 0R6
Téléphone (613) 993-2209
Télécopieur (613) 952-7656

The *Journal* is abstracted or indexed in:/Le *Journal* est résumé ou signalé dans :

Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Biological & Agricultural Index, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, Meteorological and Geoastrophysical Abstracts, Oceanic Abstracts, and Science Citation Index

Density-Dependent Juvenile Mortality in Marine Demersal Fish

Ransom A. Myers and Noel G. Cadigan

Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada

Myers, R.A., and N.G. Cadigan. 1993. Density-dependent juvenile mortality in marine demersal fish. Can. J. Fish. Aquat. Sci. 50: 1576–1590.

We examine the hypothesis that population variability is created and regulated in the juvenile stage for demersal marine fish. Juvenile mortality is examined for 17 populations of Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), plaice (*Pleuronectes platessa*), yellowtail flounder (*Pleuronectes ferrugineus*), and sole (*Solea vulgaris*) in the North Sea, Irish Sea, Barents Sea, Baltic Sea, and Northwest Atlantic. A latent variable covariance analysis of recruitment time series with measurement error is used to test hypotheses. We found strong evidence of density-dependent mortality within cohorts during the juvenile stage for cod, plaice, sole, and whiting; density-dependent mortality appears to be related to the log of juvenile abundance. There is evidence of negative autocorrelation between adjacent cohorts of cod; this pattern is consistent with density-dependent mortality between adjacent cohorts. The autocorrelations are positive for the flatfish examined. It is possible to obtain estimates of estimation error variances for populations if there are multiple research surveys of the same population. We conclude that the juvenile stage is very important for population regulation in most species but that the source of the variability in year class strength is in the larval stage or very early juvenile stage.

Nous examinons l'hypothèse que la variabilité des populations de poissons démersaux apparaît au stade juvénile et est régularisée au même stade. Nous examinons la mortalité juvénile dans 17 populations de morues franches (*Gadus morhua*), d'églefins (*Melanogrammus aeglefinus*), de merlans (*Merlangius merlangus*), de plies (*Pleuronectes platessa*), de limande à queue jaune (*Pleuronectes ferrugineus*) et de soles (*Solea vulgaris*) de la mer du Nord, de la mer d'Irlande, de la mer de Barents, de la mer Baltique et du N.-O. de l'Atlantique. Pour la vérification des hypothèses, nous nous sommes servis de l'analyse de covariance à variables latentes appliquée aux séries chronologiques du recrutement avec erreur de mesure. Nous avons constaté de forts indices que la mortalité dépend de la densité dans les cohortes du stade juvénile de la morue, de la plie, de la sole et du merlan. La mortalité dépendante de la densité semble reliée au logarithme de l'abondance des juvéniles. Il y a des signes d'autocorrélation négative entre les cohortes adjacentes de la morue; cette manifestation correspond à la mortalité dépendante de la densité entre les cohortes adjacentes. Les autocorrélations sont positives pour les poissons plats examinés. Il est possible d'obtenir des estimations de la variance des erreurs d'estimation pour les populations si la même population a fait l'objet de multiples relevés de recherche. Nous concluons que le stade juvénile est très important pour la régularisation des populations de la plupart des espèces, mais que la variabilité de l'effectif d'une classe d'âge trouve son origine au stade larvaire ou très tôt au stade juvénile.

Received December 2, 1992
Accepted April 28, 1993
(JB714)

Reçu le 2 décembre 1992
Accepté le 28 avril 1993

Our purpose is to examine density-dependent mortality within and between cohorts of marine fish during the juvenile stage. The approach taken is to consider populations for which multiple surveys are available. We use statistical methods that combine research surveys so that the underlying variability can be separated from the estimation error. We wish to identify the life-history stage in which density-dependent mortality is important. In spite of the crucial importance of this question (Ursin 1982; Shepherd and Cushing 1990), very little is known about the relative importance of density dependence in different life-history stages.

Density-dependent mortality during the juvenile stage has been most thoroughly studied in North Sea plaice (*Pleuronectes platessa*) (Rauck and Zijlstra 1978; Lockwood 1980; Veer 1986; Veer et al. 1990; Iles and Beverton 1991; Beverton and Iles 1992a, 1992b). Plaice is particularly amenable to study because the juvenile stage can be sampled. Sundby et al. (1989) presented evidence that juvenile mortality in northeast Atlantic cod (*Gadus morhua*) is also density dependent. Although these studies are on the whole convincing, it usually cannot be excluded that the negative correlation between abundance and mortality is not a statistical artifact, i.e., abundance and mortality are usually not independent variables (Veer 1986). The approach taken here eliminates this statistical artifact that has plagued previous studies.

We have tried to assemble all time series of research survey estimates in which there are at least four independent, reliable estimates of juveniles for each cohort. The major limitation of our analysis is that the time series are relatively short. Thus, the reliability of the results for any one time series is limited. However, by analyzing many populations using identical methods, it is possible to arrive at conclusions with greater reliability. We test the sensitivity of our conclusions by analyzing alternative methods of estimation and by simulations to assess the effect of small sample size and violations of the model assumptions.

TABLE 1. Data for the North Sea cod stock from VPA in millions of fish, IYFS innumbers per hour fished, and EGFS in numbers per hour fished.

Year class	VPA 1-yr-olds	IYFS 1-yr-olds	IYFS 2-yr-olds	EGFS 0-yr-olds	EGFS 1-yr-olds	EGFS 2-yr-olds
1970	847	98.30	34.50			
1971	159	4.10	10.60			
1972	289	38.00	9.50			
1973	232	14.70	6.20			
1974	426	40.30	19.90			
1975	196	7.90	3.20			4.50
1976	726	36.70	29.30		62.70	12.50
1977	426	12.90	9.30	13.90	22.80	5.80
1978	449	9.90	14.80	12.60	24.20	6.70
1979	800	16.90	25.50	18.60	50.80	13.90
1980	271	2.90	6.70	10.20	11.40	2.90
1981	557	9.20	16.60	74.20	32.40	11.00
1982	269	3.90	8.00	2.50	15.40	4.70
1983	534	15.20	17.60	95.10	61.20	11.90
1984	108	0.90	3.60	0.40	4.30	1.20
1985	581	17.00	28.80	8.30	34.40	10.70
1986	257	8.80	6.10	1.20	14.20	4.10
1987	201	3.60	6.30	0.40	8.40	2.50
1988	324	13.10	15.20	16.80	22.80	5.10
1989		3.30		6.0	6.10	
1990				3.90		

There is an important secondary outcome of our work. We obtain independent (of the sampling design) estimates of the accuracy of abundance estimates obtained from research surveys.

In this paper, we examine only the component of juvenile mortality related to population abundance, i.e., the density-dependent component. For populations that have two independent, simultaneous surveys of population abundance, it is possible to estimate the variability of juvenile mortality unrelated to population abundance. This is done in Myers and Cadigan (1993).

An Example

Before presenting the statistical model, it is useful to discuss an example first. In Table 1, we show the results, presented to the ICES assessment working group (ICES 1991a), from research trawl surveys of cod in the North Sea. The English Groundfish Surveys (EGFS) take place in the autumn, while the International Young Fish Survey (IYFS) takes place in the late winter (February–March). Also included is the virtual population analysis (VPA) of the population (Pope 1972). Several things are immediately apparent from the data:

(1) Large and small year classes are consistent through all the surveys and the VPA.

(2) There is much greater variation in large and small year classes at early ages than at later ages. This appears not to be due to estimation error alone because the small and large year classes show up consistently throughout all the analyses.

(3) There is much greater variation in the research surveys than in the VPA.

(4) There appears to be density-dependent mortality, i.e., large year classes appear to have higher mortality. To illustrate this, we plot an index of survival (the difference between the log numbers at age 2 and 0 from the EGFS) against an independent index of abundance of that cohort (the log numbers at age 1 from the EGFS). There appears to be a significant decrease in survival with log abundance. The estimation errors for the two variables in Fig. 1 are independent, and thus the negative slope is not caused by correlated errors.

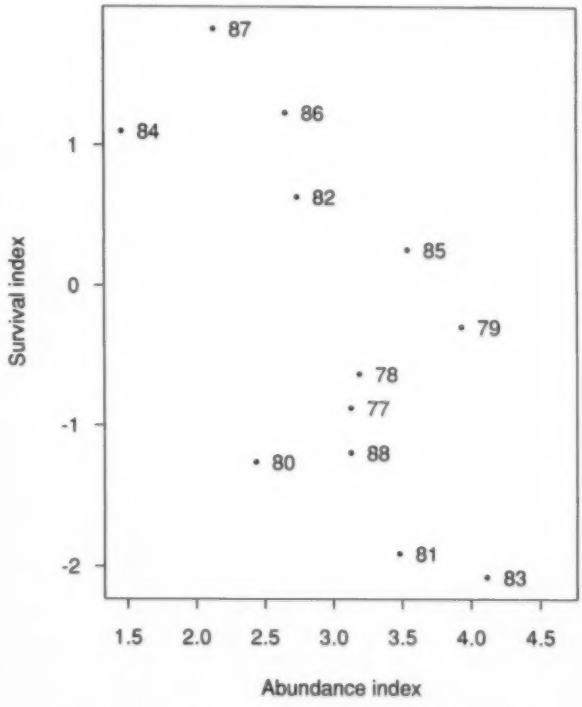


FIG. 1. Survival index versus abundance index for the EGFS of North Sea cod. The index of survival is the difference between the log index of numbers at age 2 and 0. The index of abundance is the log numbers at age 1. The correlation is −0.65 (p-value = 0.0054).

(5) Recruitment appears to be low after large cohorts. This implies that there may be density-dependent mortality between adjacent cohorts.

In this paper, we examine the above points using stochastic models.

Methods

The Basic Model

Consider a population in which each cohort is surveyed several times in the juvenile stage. Let the number of fish in cohort t surveyed at age a be $N_{t,a}$. The surveys see the juveniles at different ages; the survey index of the youngest age is 0, and surveys that see the fish at later ages are labeled sequentially. Let the average mortality between the first survey and the survey at age a that is independent of density be m_a. The population dynamics during the juvenile stage are described by

$$N_{t,a} = N_{t,0}\, e^{-m_a}.$$

Here, $m_0 = 0$.

Now consider the case of density-dependent mortality. We examine the hypothesis that mortality is linearly related to the logarithm of the initial number of juveniles in the cohort (Fig. 1). Let $(1-\lambda_a)\log N_{t,0}$ be the density-dependent mortality where $\lambda_0 = 1$. We now have that

$$N_{t,a} = N_{t,0}\, e^{-m_a-(1-\lambda_a)\log N_{t,0}}$$

Note that the density-dependent component of mortality is a function of the numbers of fish in the cohort at the first time they are seen in the surveys.

It is useful to work with log-transformed variables, e.g., $L_{t,a} = \log N_{t,a}$. Thus, the above equation becomes

$$L_{t,a} = \lambda_a L_{t,0} - m_a$$

Consider observational error in the log-transformed survey data, $l_{t,a,i}$. Let $c_{a,i}$ be the log catchability of survey i on age a and let $\delta_{t,a,i}$ be the observational error of survey i on age a of cohort t. Estimates of abundance are assumed to be proportional to the true abundance. We write $\lambda_{a,i}$ now instead of λ_a. The observed log-transformed abundance from age a and survey i is

$$(1)\quad l_{t,a,i} = c_{a,i} + L_{t,a} + \delta_{t,a,i}$$

$$= c_{a,i} - m_a + \lambda_{a,i} L_{t,0} + \delta_{t,a,i}.$$

The following assumptions are made about the stochastic nature of recruitment and estimation errors when estimating the parameters of Eq. 1:

(1) Relative year class strength is determined in the larval stage, and there is no interannual variation in mortality in the juvenile stage unrelated to density. This assumption is tested in Myers and Cadigan (1993).

(2) Log-transformed standardized recruitments are mutually independent, identically distributed random variables (i.e., independent of year) following a normal distribution with constant mean and variance, that is

$$L_{t,0} \sim N(\mu, \phi).$$

(3) Errors in estimating abundance from research surveys, $\delta_{t,a,i}$, are independent normal random variables with zero mean and constant variance, that is

$$\delta_{t,a,i} \sim N(0, \theta_{a,i}).$$

Furthermore, the errors are independent of $L_{t,0}$. The variance is constant in that it does not depend upon year, but it does depend upon age and survey.

Note that $c_{a,i} - m_a$ is constant for each survey and age. We do not attempt to estimate these terms, but merely subtract the sample mean over time for each survey and age from the data. We concentrate on estimating the other parameters in Eq. 1.

Density-dependent mortality may also be a function of the numbers in the previous cohort abundance, $N_{t-1,0}$. In this case, we define between cohort density-dependent mortality such that

$$N_{t,0} = N_{t,l}\, e^{\beta \log N_{t-1,0}}.$$

$N_{t,l}$ is defined as the recruitment before the effects of density-dependent mortality have occurred. The subscript l refers to the larval stage. Taking logarithms yields

$$(2)\quad L_{t,0} = L_{t,l} + \beta L_{t-1,0}.$$

We assume that $L_{t,l}$ is independent of $L_{t-1,0}$. We also assume that $L_{t,0}$ is normal with constant variance. If the variance of $L_{t,0}$ is ϕ, as we assumed before, then the variance of $L_{t,l}$ is $(1-\beta^2)\phi$.

In Eq. 2, adjacent cohorts are assumed to interact through increased mortality that occurs before the first juvenile survey. However, the results from many VPA estimates of recruitment are positively autocorrelated (Myers et al. 1990). This may be because recruitment is responding to autocorrelated environmental variable or because errors in aging commercial catch at age data used by VPA's induce autocorrelated errors (Myers and Drinkwater 1989; Bradford 1991). We eliminate the second problem by using only research vessel data on juvenile fish in which aging is thought to be very accurate because there is minimal size overlap between the youngest ages. Thus, the parameter β can be interpreted as the density-dependent mortality between adjacent cohorts, or as the autocorrelation between the log of adjacent cohorts.

When estimating β in Eq. 2 the above assumptions must be modified. Specifically, we modify the assumption that recruitment is independent between adjacent cohorts. We estimate the parameter β by estimating the covariance between the factors describing recruitment in year t and $t-1$. In order to estimate β, we must examine the assumption that the errors in estimation are independent between surveys. A likely source of nonindependent errors occurs because the errors in estimating abundance of all ages in a survey often appear to be positively correlated. When the same survey is used to estimate the numbers at two age groups and lagged data are used, this could create spurious correlations. Let $l_{t,1,i}$ be an estimate of recruitment from survey i for age 1, and let $l_{t-1,2,i}$ be the estimate for age 2 lagged 1 yr from the same survey. Then $l_{t,1,i}$ and $l_{t-1,2,i}$ both are estimated during the same year, and it is not reasonable to assume that the estimation errors are independent. That is, the covariance between the estimation errors, i.e., cov $(\delta_{t,1,i}, \delta_{t-1,2,i})$, is positive. We estimate all such covariance parameters in the model. We present the estimated correlation, e.g., $\rho(\delta_{t,1,i}, \delta_{t-1,2,i})$ instead of the covariance because it is easier to understand. Note that because the estimated correlation is derived from covariance estimates, it may sometimes be estimated as being greater than 1. We present

TABLE 2. Parameters and estimates for the density dependence between cohorts model, North Sea (SE's in parentheses), ρ (·) denotes the correlation coefficient; *p*-values are two-sided probabilities that estimates are not zero; $P(\lambda > 1)$ is the joint probability that all the estimated λ's are > 1.

	Cod	Haddock	Whiting		Plaice	Sole
$l_{t,0,1}$	EGFS	EGFS	EGFS	$l_{t,0,1}$	INSHORE	INSHORE
$l_{t,1,2}$	IYFS	IYFS	IYFS	$l_{t,0,2}$	TRIDENSA	TRIDENSA
$l_{t,1,1}$	EGFS	EGFS	EGFS	$l_{t,1,3}$	TRIDENSS	TRIDENSS
$l_{t,2,2}$	IYFS	IYFS	IYFS	$l_{t,1,2}$	TRIDENSA	TRIDENSA
$l_{t,2,1}$	EGFS	EGFS	EGFS	$l_{t,1,1}$	INSHORE	INSHORE
n	18	18	18	n	21	21
$\lambda_{0,1}$	1.00	1.00	1.00	$\lambda_{0,1}$	1.00	1.00
$\lambda_{1,2}$	0.50	0.68	0.55	$\lambda_{0,2}$	1.00	1.00
	(0.07)	(0.12)	(0.23)			
$\lambda_{1,1}$	0.47	0.88	0.70	$\lambda_{1,3}$	0.69	0.72
	(0.06)	(0.12)	(0.18)		(0.11)	(0.11)
$\lambda_{2,2}$	0.40	0.68	0.26	$\lambda_{1,2}$	0.60	0.22
	(0.06)	(0.12)	(0.23)		(0.08)	(0.13)
$\lambda_{2,1}$	0.45	1.0	0.41	$\lambda_{1,1}$	0.57	0.52
	(0.06)	(0.15)	(0.17)		(0.11)	(0.13)
$\theta_{0,1}$	0.99	0.39	0.31	$\theta_{0,1}$	0.15	0.10
	(0.30)	(0.12)	(0.10)		(0.05)	(0.12)
$\theta_{1,2}$	0.13	0.15	0.29	$\theta_{0,2}$	0.16	0.91
	(0.04)	(0.05)	(0.08)		(0.06)	(0.23)
$\theta_{1,1}$	0.02	0.07	0.01	$\theta_{1,3}$	0.30	0.58
	(0.01)	(0.03)	(0.02)		(0.07)	(0.14)
$\theta_{2,2}$	0.05	0.16	0.36	$\theta_{1,2}$	0.18	0.90
	(0.02)	(0.05)	(0.10)		(0.05)	(0.20)
$\theta_{2,1}$	0.01	0.12	0.15	$\theta_{1,1}$	0.28	1.06
	(0.01)	(0.05)	(0.04)		(0.07)	(0.24)
$\rho(\delta_{t,0,1}, \delta_{t-1,1,1})$	0.48	0.28	–0.4	$\rho(\delta_{t,0,1}, \delta_{t-1,1,1})$	0.01	1.00
p-value	(0.12)	(0.43)	(0.49)	*p*-value	(0.96)	(0.02)
$\rho(\delta_{t,1,1}, \delta_{t-1,2,1})$	1.06	–0.14	0.60	$\rho(\delta_{t,0,2}, \delta_{t-1,1,2})$	0.56	0.22
p-value	(0.02)	(0.73)	(0.33)	*p*-value	(0.04)	(0.31)
$\rho(\delta_{t,1,2}, \delta_{t-1,2,2})$	0.50	–0.50	0.48			
p-value	(0.08)	(0.07)	(0.06)			
ϕ	2.69	0.93	0.27	ϕ	0.78	1.45
	(1.15)	(0.36)	(0.15)		(0.21)	(0.35)
β	–0.59	0.19	–0.68	β	0.43	0.12
p-value	(0.12)	(0.52)	(0.16)	*p*-value	(0.11)	(0.61)
$P(\lambda > 1)$	0.000	0.508	0.061	$P(\lambda > 1)$	0.002	0.006

such estimates but make no inferences about them.

Using the above assumptions, it is possible to obtain maximum likelihood estimates of the model parameters. We defer more discussion of parameter estimation to Appendix 1.

Many readers may suspect that estimating density-dependent mortality from research surveys is not possible because of large biases due to the errors-in-variable regression problem (Hilborn and Walters 1992). We have addressed this problem using Monte Carlo simulations and have found that such biases do not appear to be a problem for our methods (Appendix 2).

Data

We examined data from regions where there are multiple surveys in each year and used the following criteria for selecting time series. First, we attempted to use surveys that covered the complete range of the population. Unfortunately, this was not always possible. Some surveys covered regions close to shore, while others, such as the North Wales Groundfish Survey (NWGS), covered only a portion of the region. We have identified such limitations of the data below. Second, we had to deal with the problem of zero catches in some years. We attempted to use surveys that only had nonzero catches. If there were zero catches, we replaced the observation with one tenth the minimum of the nonzero catches. We performed the above procedure with one half the minimum and found that estimates were not very sensitive to the fraction of the minimum. The data from some surveys appeared to have so much estimation error that estimates were unrealistic. In analyzing the robustness of the methodology (Appendix 2), estimates from such surveys were considerably biased and had large standard errors. We have identified these cases below.

The North Sea gadoid populations were estimated from two surveys that cover the area very well (ICES 1991a). The EGFS (Table 2) take place in the autumn, while the IYFS takes place in the late winter (February–March). The eastern North Sea populations of sole (*Solea vulgaris*) and plaice were surveyed during the spring (April–May) and autumn (September–October) from the Dutch to the Danish coastal waters using the research vessel *Tridens* (ICES 1991b). The surveys are denoted by TRIDENSA and TRIDENSS for the age 0+ and 1+ surveys in the autumn and the age 1+ surveys carried out in the spring (Table 2). These surveys have been carried out since 1970. There are surveys carried out at approximately the same time in more

TABLE 3. Parameters and estimates for the density dependence between cohorts model, Irish Sea (SE's in parentheses). See Table 2 for details.

Cod		Whiting		Plaice	
$l_{t,0,1}$	OCTOBER	$l_{t,0,1}$	GADOID	$l_{t,0,1}$	OCTOBER
$l_{t,1,2}$	MARCH	$l_{t,0,2}$	OCTOBER	$l_{t,1,2}$	MARCH
$l_{t,1,3}$	JUNE	$l_{t,1,3}$	JUNE	$l_{t,1,3}$	JUNE
$l_{t,1,1.}$	OCTOBER	$l_{t,1,1.}$	GADOID	$l_{t,1,4}$	ISMAY
n	14	n	11	n	14
$\lambda_{0,1}$	1.00	$\lambda_{0,1}$	1.00	$\lambda_{0,1}$	1.00
$\lambda_{1,2}$	0.75	$\lambda_{0,2}$	1.00	$\lambda_{1,2}$	0.93
	(0.12)				(0.23)
$\lambda_{1,3}$	0.51	$\lambda_{1,3}$	0.45	$\lambda_{1,3}$	(0.52)
	(0.11)		(0.30)		0.18
$\lambda_{1,1}$	0.65	$\lambda_{1,1}$	0.82	$\lambda_{1,4}$	0.26
	(0.13)		(0.38)		(0.13)
$\theta_{0,1}$	0.56	$\theta_{0,1}$	0.74	$\theta_{0,1}$	1.05
	(0.36)		(0.34)		(0.48)
$\theta_{1,2}$	0.58	$\theta_{0,2}$	1.15	$\theta_{1,2}$	0.20
	(0.25)		(0.46)		(0.32)
$\theta_{1,3}$	0.78	$\theta_{1,3}$	0.44	$\theta_{1,3}$	0.88
	(0.26)		(0.17)		(0.29)
$\theta_{1,1}$	0.89	$\theta_{1,1}$	0.42	$\theta_{1,4}$	0.55
	(0.31)		(0.22)		(0.17)
$\rho(\delta_{t,0,1}, \delta_{t-1,1,1})$	0.57	$\rho(\delta_{t,0,1}, \delta_{t-1,1,1})$	–0.04		
p-value	(0.17)	p-value	(0.92)		
ϕ	3.72	ϕ	0.58	ϕ	1.93
	(1.32)		(0.36)		(0.93)
β	–0.24	β	0.20	β	0.26
p-value	(0.48)	p-value	(0.68)	p-value	(0.47)
$P(\lambda > 1)$	0.019	$P(\lambda > 1)$	0.338	$P(\lambda > 1)$	0.382

inshore regions from the Belgium to the Danish coastal waters. These are autumn surveys (September–October) that are primarily aimed at estimating the numbers of 0+ recruits. These surveys are denoted by INSHORE0 and INSHORE1 for the age 0+ and 1+ fish.

The Irish Sea gadoid populations are estimated from the October, March, and June NWGS (Table 3) and the autumn (September–October) prerecruit gadoid surveys, denoted as GADOID (Brander and Symonds 1981; ICES 1991c). The NWGS take place at 16 fixed sites along the North Wales coast, and even though the coverage is only of a portion of the ranges of the populations, they are highly correlated with recruitment calculated from VPA (ICES 1991c). These surveys have taken place every year since 1976. The prerecruit gadoid survey is a 32-haul survey stratified by depth throughout the Irish Sea. This survey occurred from 1979 to 1987. The Irish Sea plaice population is also surveyed by the Irish May Survey, which took place from 1976.

Random stratified surveys of the Gulf of Maine, Georges Bank, and southern New England) populations have taken place in the offshore regions by the U.S. Northeast Fisheries Center (NEFC) in the spring since 1968 and in the fall since 1963 (Table 4). Abundance is estimated with few or no zero catches at age 1+ in the spring surveys (Anonymous 1991). Cod, haddock (*Melanogrammus aeglefinus*), yellowtail flounder (*Pleuronectes ferrugineus*), and silver hake (*Merluccius bilinearis*) have been aged in these surveys and are thus amenable for our analysis; however, the errors in estimating the numbers of silver hake were too large to obtain reliable estimates.

Surveys of cod on the southern Grand Banks of Newfoundland (Table 5) have taken place since 1972 by Canada (Baird et al. 1991) and since 1978 by the USSR (Kuzmin 1991). Both surveys are in the spring and cover the same region. However, the Russian surveys did not appear to give reliable estimates until age 3. There are surveys by Canada and France on the nearby St. Pierre Bank; however, the estimation errors for juveniles were too large to produce reliable results.

The Northeast Arctic haddock population has been surveyed in the Barents Sea by Norway using trawl surveys, denoted by NORWAYB, since 1983 and acoustic surveys, denoted by NORWAYA, since 1977 during winter (January–March) with only one year missing for the acoustic survey (Table 5). Young age groups of Northeast Arctic cod do not seem to be as well surveyed by the acoustic surveys; there are several years in which no age 1+ cod were caught. We therefore used the USSR bottom trawl surveys carried out during late autumn – early winter (November–December) which did not record zero catches for any juvenile age class (ICES 1991d and unpublished data). For the USSR trawl surveys for cod, we use the combined index for ICES subareas I, IIa, and IIb.

Survey Reliability

Two crucial assumptions in the analysis are that the research survey estimates of population abundance are proportional to the true abundance and that the populations are well defined. Estimates of abundance from a well-designed research survey should respond proportionally to changes in true population abundance if the total range of the population is surveyed. In most of the surveys considered here, this is the case. However, even if only a portion of the range is surveyed the estimates may be proportional to the true abundance.

TABLE 4. Parameter and estimates for the density dependence between cohorts model, United States (SE's in parentheses). See Table 2 for details.

	Cod Georges Bank	Cod Gulf of Maine	Haddock Georges Bank	Haddock Gulf of Maine	Yellowtail Georges Bank	Yellowtail Southern New England
$l_{t,1,1}$	SPRING	SPRING	SPRING	SPRING	SPRING	SPRING
$l_{t,1,2}$	FALL	FALL	FALL	FALL	FALL	FALL
$l_{t,2,1}$	SPRING	SPRING	SPRING	SPRING	SPRING	SPRING
$l_{t,2,2}$	FALL	FALL	FALL	FALL	FALL	FALL
n	26	26	26	26	26	26
$\lambda_{1,1}$	1.00	1.00	1.00	1.00	1.00	1.00
$\lambda_{1,2}$	1.08	0.79	0.91	1.06	0.88	0.94
	(0.17)	(0.15)	(0.09)	(0.12)	(0.19)	(0.19)
$\lambda_{2,1}$	1.10	0.71	0.73	1.01	0.78	1.06
	(0.18)	(0.15)	(0.07)	(0.09)	(0.13)	(0.18)
$\lambda_{2,2}$	0.88	0.53	0.71	1.05	0.60	1.18
	(0.16)	(0.12)	(0.09)	(0.10)	(0.12)	(0.23)
$\theta_{1,1}$	0.74	1.99	1.71	1.55	3.00	2.54
	(0.16)	(0.46)	(0.48)	(0.39)	(0.68)	(0.53)
$\theta_{1,2}$	0.19	0.74	2.02	2.31	1.31	1.05
	(0.06)	(0.19)	(0.51)	(0.55)	(0.33)	(0.24)
$\theta_{2,1}$	0.10	0.59	0.71	0.60	0.51	0.57
	(0.05)	(0.16)	(0.22)	(0.22)	(0.16)	(0.15)
$\theta_{2,2}$	0.26	0.57	2.31	1.17	0.25	0.29
	(0.06)	(0.13)	(0.52)	(0.33)	(0.08)	(0.13)
$\rho(\delta_{t,1,1}, \delta_{t-1,2,1})$	0.79	0.87	0.05	−0.04	0.70	0.89
p-value	(0.00)	(0.00)	(0.86)	(0.89)	(0.49)	(0.00)
$\rho(\delta_{t,1,2}, \delta_{t-1,2,2})$	0.36	0.78	0.82	0.77	0.31	0.71
p-value	(0.13)	(0.00)	(0.00)	(0.00)	(0.76)	(0.00)
ϕ	0.68	1.45	7.33	6.09	2.37	1.18
	(0.24)	(0.55)	(1.89)	(1.71)	(1.01)	(0.51)
β	−0.22	−0.00	−0.14	0.45	0.76	0.46
p-value	(0.33)	(1.00)	(0.55)	(0.07)	(0.45)	(0.14)
$P(\lambda > 1)$	0.784	0.085	0.164	0.836	0.257	0.821

Myers and Stokes (1989) examined the EGFS in the North Sea to determine if local population log abundance changed uniformly throughout the North Sea. They found that this was true for haddock, but not for cod and whiting (*Merlangius merlangus*). There are few instances where this needs to be considered in detail. In the plaice and sole survey on the continental side of the North Sea, we used two independent surveys, each of which covers a portion of the habitat. Jointly, these surveys cover much of the range of the population, and the estimates should be reliable because we have constrained both estimates to be proportional to each other, i.e, we have constrained both $\lambda_{0,1}$ and $\lambda_{0,2}$ to be 1.

The U.S. surveys do not cover the nearshore areas. This may present a problem for Gulf of Maine cod which has its juvenile nursery grounds close to shore. A portion of the inshore area is covered by the State of Massachusetts Inshore Surveys. In Myers and Cadigan (1993), we estimated density-dependent mortality using these data and found significant density-dependent mortality in this population whereas in the current analysis (Table 2), we did not.

In the Irish Sea, the NWS covers only a portion of the range of the populations. Thus the results for this region must be viewed with some reservation.

For any one of the above populations, density-dependent habitat utilization combined with surveys that do not cover the entire range of the populations could bias the results. In particular, if only the core area of the population was surveyed, density-dependent habitat utilization might appear as density-dependent mortality. We conclude that this factor may be important in a few of the populations, but should have only limited influence on our overall conclusions.

Results

Density-Dependent Mortality within a Cohort

We initially estimated the model under the assumption that recruitment was independent between adjacent year classes ($\beta = 0$). We then estimated the model without the above assumption. Our results were similar in both cases. To save space, we present only the results for the case without the assumption because the parameter estimates (other than β, Tables 3–5) are very similar.

If there is density-dependent mortality within cohorts, we expert $\lambda_a < 1$ for older ages. For cod, λ_a decreases with age for all populations except the Georges Bank population (Fig. 2). This implies that the variability in recruitment is being reduced through density-dependent mortality or a process similar to density dependent mortality. Georges Bank cod is perhaps the most overexploited of the cod populations (Anonymous 1991). It may show no evidence of density-dependent mortality because the population abundance has never reached a level where density-dependent mortality would be important.

The density-dependent mortality parameter, λ, appears to be typically around 0.5 for cod. This would imply that a 100-fold increase in postlarval cod would result in only a 10-fold increase in abundance a year later. There appears to be strong density-

TABLE 5. Parameters and estimates for the density dependence between cohorts model, Newfoundland and Arctic (SE's in parentheses). See Table 2 for details.

	Cod Southern Grand bank		Cod Northeast Arctic	Haddock Northeast Arctic
$l_{t,1,1}$	CANADA	$l_{t,1,1}$	RUSSIA	NORWAYA
$l_{t,2,1}$	CANADA	$l_{t,1,2}$	NORWAYB	NORWAYB
$l_{t,3,1}$	CANADA	$l_{t,2,1}$	RUSSIA	NORWAYA
$l_{t,3,2}$	RUSSIA	$l_{t,2,2}$	NORWAYB	NORWAYB
n	18	n	9	11
$\lambda_{1,1}$	1.00	$\lambda_{1,1}$	1.00	1.00
$\lambda_{2,1}$	0.60	$\lambda_{1,2}$	0.87	0.81
	(0.15)		(0.17)	(0.24)
$\lambda_{3,1}$	0.45	$\lambda_{2,1}$	0.67	1.08
	(0.12)		(0.13)	(0.30)
$\lambda_{3,2}$	0.60	$\lambda_{2,2}$	0.57	0.97
	(0.18)		(0.12)	(0.29)
$\theta_{1,1}$	2.73	$\theta_{1,1}$	2.27	2.99
	(0.82)		(0.95)	(1.27)
$\theta_{2,1}$	0.18	$\theta_{1,2}$	0.80	1.33
	(0.13)		(0.43)	(0.62)
$\theta_{3,1}$	0.40	$\theta_{2,1}$	0.64	1.66
	(0.13)		(0.30)	(0.90)
$\theta_{3,2}$	0.97	$\theta_{2,2}$	0.53	1.89
	(0.29)		(0.24)	(0.89)
$\rho(\delta_{t,1,1}, \delta_{t-1,2,1})$	0.68	$\rho(\delta_{t,1,2}, \delta_{t-1,2,1})$	0.36	0.01
p-value	(0.07)	p-value	(0.39)	(0.98)
$\rho(\delta_{t,2,1}, \delta_{t-1,3,1})$	0.47	$\rho(\delta_{t,1,2}, \delta_{t-1,2,2})$	0.45	0.12
p-value	(0.24)	p-value	(0.32)	(0.98)
ϕ	2.28	ϕ	5.69	4.18
	(1.15)		(2.89)	(2.53)
β	–0.06	β	0.49	0.53
p-value	(0.85)	p-value	(0.26)	(0.28)
$P(\lambda > 1)$	0.015	$P(\lambda > 1)$	0.216	0.697

dependent mortality for sole and plaice. These observations are consistent with previous observations of density-dependent mortality in plaice from the analysis of other types of data. The results for yellowtail flounder are ambiguous. The Georges Bank population shows evidence of density-dependent mortality whereas the southern New England population does not.

Both populations of whiting show density-dependent mortality. Density-dependent mortality in haddock appears to be less important than in the two other gadoids considered. However, all haddock populations except Gulf of Maine show some evidence of density-dependent mortality.

Overall, there appears to be generally very high levels of density-dependent mortality in most populations examined. The consistency of the relationship implies that it is a general phenomenon. The results are robust to reasonable violations of the model assumptions (Appendix 2).

Density-Dependent Mortality between Cohorts

To test the assumption that recruitment is independent between adjacent cohorts, we estimated the parameter β, which is the first-order autocorrelation between cohorts. We did this by estimating the covariance between the factors describing recruitment and lag recruitment (Tables 2–5). Although the individual estimates of β are not statistically significant, consistent patterns are evident.

β is positive for all the flatfish and the haddock populations. For whiting, β is slightly positive in the Irish Sea and negative in the North Sea. For cod, β is negative for five of the six populations examined. This is consistent with the hypothesis that there is density-dependent mortality between adjacent cohorts. For cod, the only exception is the Northeast Arctic population. The standard errors of the estimates of β are relatively large. Nevertheless, the strong pattern within the taxa indicates that density-dependent mortality between adjacent cohorts of cod may be a real phenomenon.

The autocorrelation between year classes is probably governed by two competing factors. The first is a negative correlation caused by density-dependent mortality due to competition and cannibalism between adjacent cohorts. The second is a positive correlation caused by autocorrelation in the environment. In some cases, i.e., Northeast Arctic cod, the second factor may dominate whereas the first factor may dominate for other populations.

Estimation Error Variances

There is a striking difference in the estimation error variance in different surveys (Fig. 3; Tables 2–5). The North Sea surveys for cod, whiting, and haddock have the smallest estimation error variance of all surveys. In particular, these surveys are much less variable than those conducted off New England, Canada, and in the Northeast Arctic. The relatively low estimation error for the North Sea surveys may be because these surveys usually employed fixed stations in contrast with the New England and Canadian stratified random surveys where rerandomization is

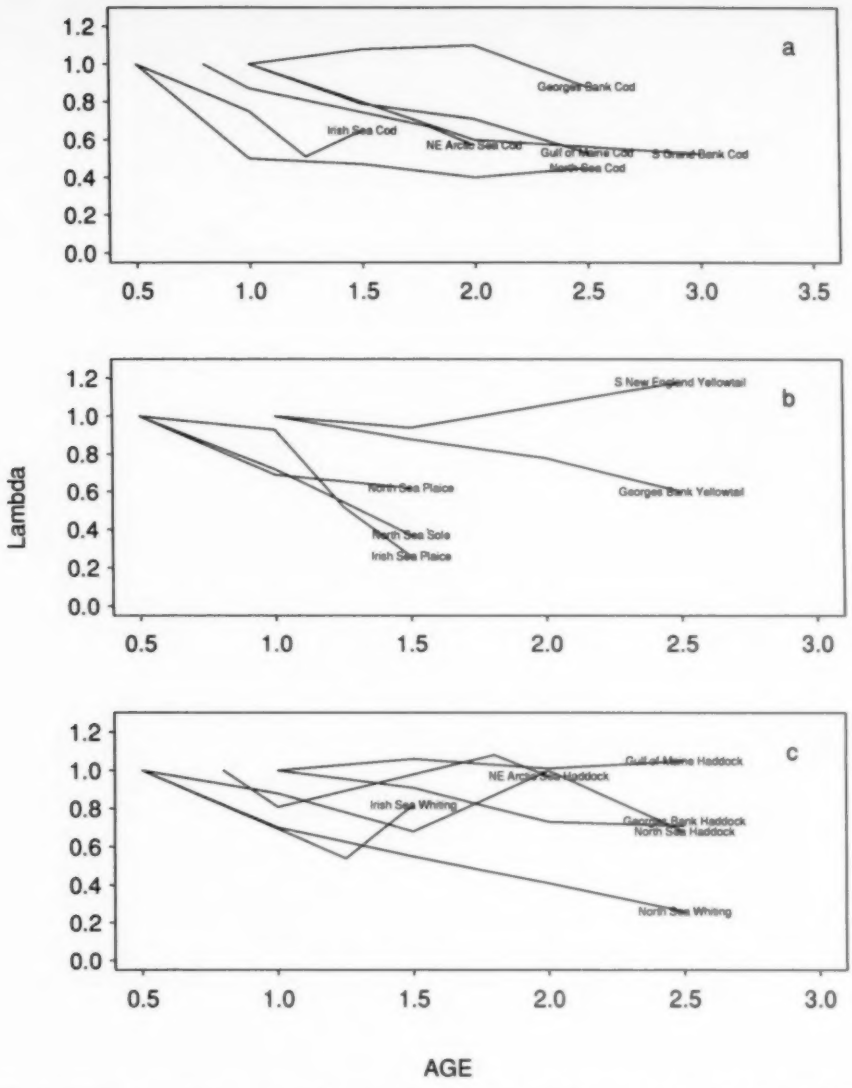


FIG. 2. Density-dependent mortality coefficient, λ, in relation to age for (a) cod, (b) plaice, sole, and yellowtail flounder, and (c) haddock and whiting.

performed each year. Note that the Irish Sea surveys have relative low estimation error variance even though these surveys have few sample trawls, i.e., either 16 or 32. The Irish sea surveys may owe their low estimation error to having fixed sample sites.

The estimation error usually decreases with age (Fig. 3). The source of the higher estimation error may be caused by the behavior of the younger fish. For example, younger fish may be more pelagic or may tend to school more, either of which would increase the sampling variability.

Note that cov $(\delta_{i,j}, \delta_{i-1,j+1,j})$ > 0 for 28 of 33 covariances estimated. Given the shortness of the series, this is good evidence of year effects in the surveys and that the standard errors calculated from survey design models will underestimate the true estimation errors. This underestimation probably occurs because the efficiency of the sampling gear is not constant. For example, environmental conditions or differences in how the crew handle the fishing gear may affect the efficiency.

Density-Dependent Mechanisms

Our results are consistent with density-dependent mortality for most of the populations examined. There are several biological processes that could result in density-dependent mortality:

(1) Density-dependent mortality could be the consequence of competition for food. Mortality may result from starvation or from increased predation, i.e., fish with reduced specific growth rates would be exposed to predators for a longer time (Ricker and Foerster 1948; Shepherd and Cushing 1980). Density-dependent growth would usually be seen if there is increased competition for food; however, competition for food may increase mortality

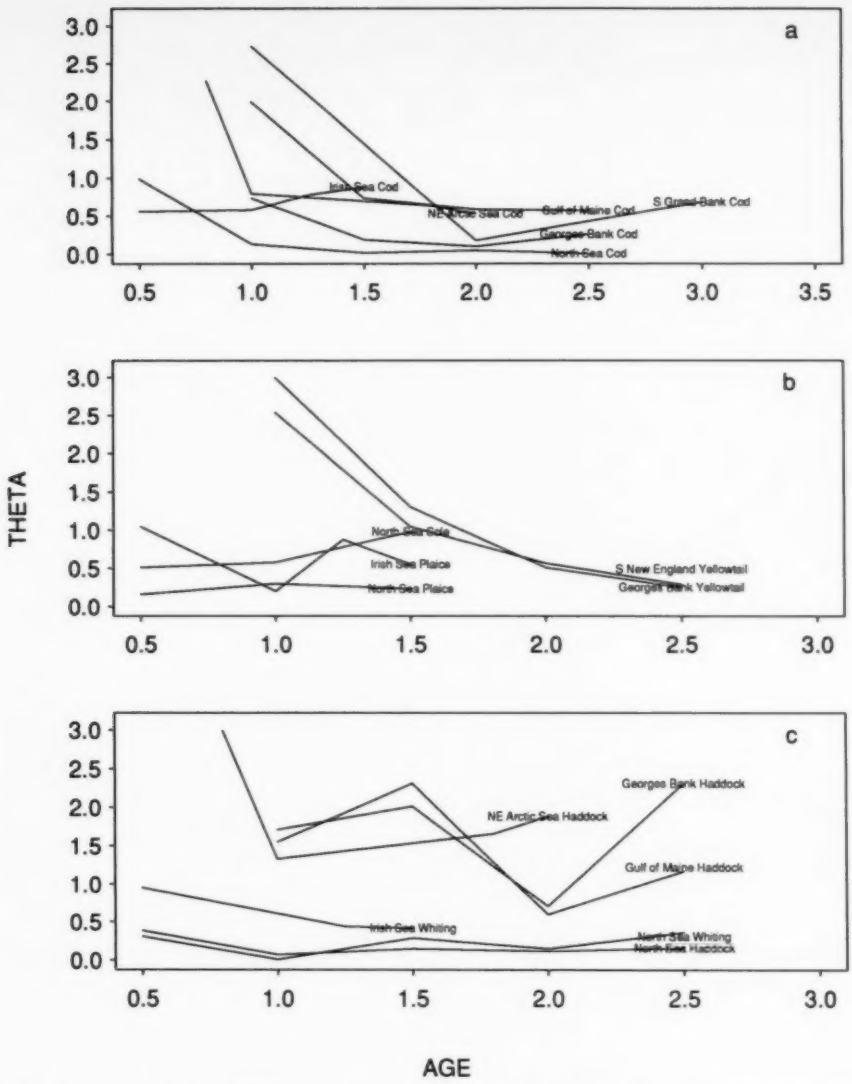


FIG. 3. Estimates of survey error variance, $\theta_{a,i}$, in relation to age for (a), cod, (b) plaice, sole, and yellowtail, and (c) haddock and whiting.

even if a change in growth rate is not seen. For example, at higher densities, fish may have to spend more time feeding and moving, which may increase exposure to predator.

(2) Cannibalism is a possible explanation for some piscivorous species such as cod and whiting. However, no evidence we know of suggests that cannibalism within a year class is an important source of mortality.

(3) Prey switching by predators to abundant year classes is a possible explanation that would be applicable to most species.

(4) Density-dependent mortality might result if there is only a fixed area of juvenile habitats, and the juveniles that settle there survive, while the others do not. In this case, a relatively fixed number of juveniles survive no matter what number was originally produced. This mechanism has been proposed to account for the relative small variation in recruitment seen in North Sea plaice (Ursin 1982). This mechanism may involve food limitation or increased predation risk outside a nursery area; it assumes that there is a mechanism to maintain a relatively constant density within the favorable habitat, e.g., territorial behavior or some other form of density-dependent habitat utilization.

Density-Dependent Fishing Mortality

The above biological hypotheses cannot be distinguished with the present data. However, it is conceivable that there is density-dependent fishing mortality on young fish. If fishermen act in the same way as prey-switching predators which target abundant year classes, then this could result in the observed patterns. If fishermen set trawls when they detect fish on their sonar fish detectors, then these schools of young fish from abundant year classes would be preferentially caught. They also might largely be discarded. Conser et al. (1992) has found that

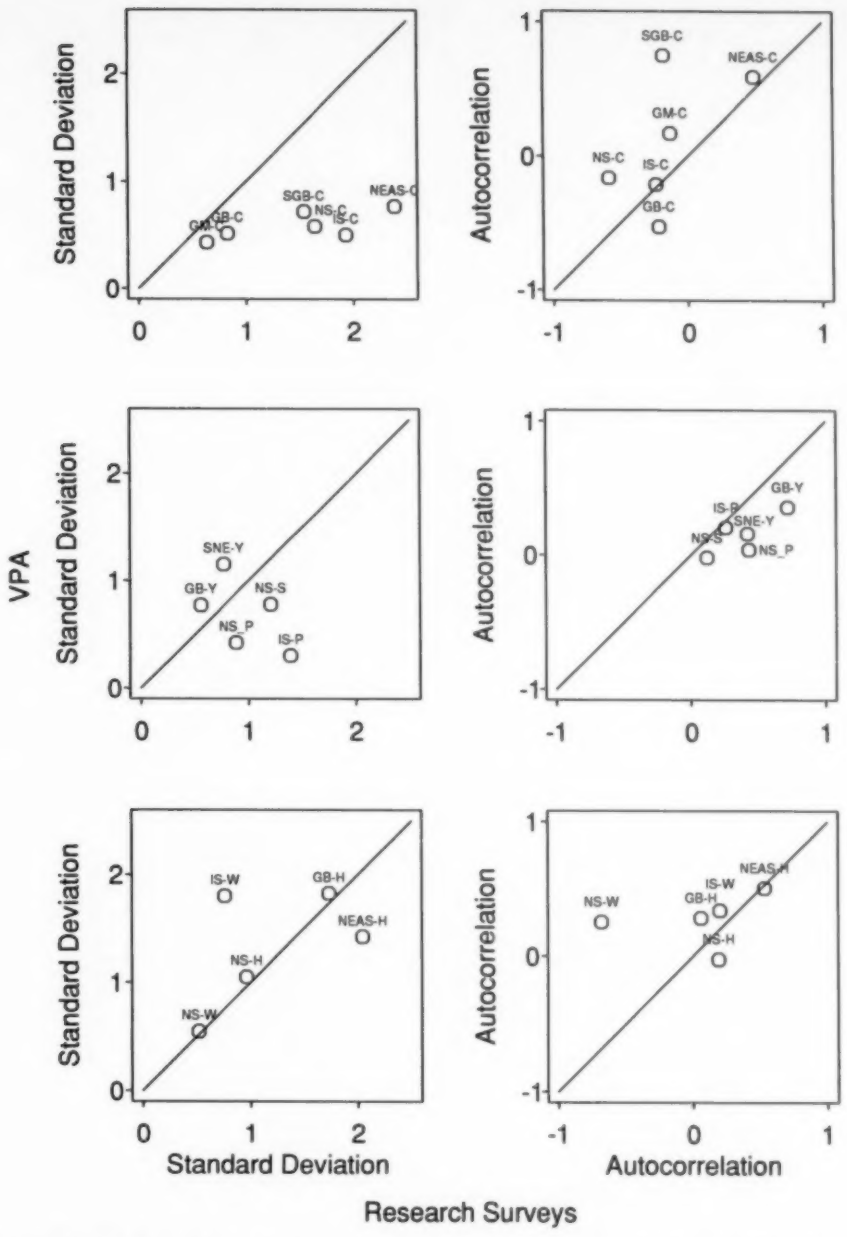


FIG. 4. Plots of estimated standard deviations of log recruitment from VPA's versus those estimated at the earliest age of the research survey used in the analysis. On the right is the first-order autocorrelation of log recruitment from VPA estimates and those obtained here (Tables 2–5). The population locations are abbreviated as follows: GB, Georges Banks; GM, Gulf of Maine; IS, Irish Sea; NS, North Sea; NEAS, Northeast Arctic Sea; SGB, southern Grand Banks; SNE, southern New England. The population names are abbreviated as follows: C, cod; H, haddock; P, plaice; S, sole; W, whiting; Y, yellowtail flounder. for example, IS-C is the Irish Sea cod population. The top panels show the results for cod, the middle panels for plaice, sole, and yellowtail flounder, and the bottom panels for haddock and whiting. The one-to-one reference lines are also shown.

discard rates of juveniles may increase with the strength of year class. If the density-dependent mortality is caused by fishing, it is then possible that production could be greatly improved if fishing pressure on young fish were reduced.

Discussion and Conclusions

This study is the first to demonstrate the general importance of density-dependent juvenile mortality in marine demersal fish. Our study confirms the results of density-dependent mortality in

plaice that have been conducted on a very restricted spatial-scale (Rauck and Zijlstra 1978; Lockwood 1980; Veer 1986; Veer et al. 1990; Iles and Beverton 1991; Beverton and Iles 1992a, 1992b).

An important result in our analysis is that density-dependent juvenile mortality appears to be significant in most of the populations we have examined. For example, haddock is usually characterized by having much more variable recruitment than cod (Ursin 1982; Myers et al. 1990; Myers 1991). However, our analysis of the North Sea and Barents Sea data demonstrates that this is true only for the postjuvenile stage. In both cases, the recruitment variability at the youngest age observed (ϕ) is greater for cod than for haddock, but density-dependent is mortality much stronger in cod so that this initial variability is greatly reduced.

Our results appear to be robust in that our conclusion that density-dependent mortality is important is not affected by reasonable violations of the model assumptions (Appendix 2). If anthing, our methods underestimate the strength of density-dependent mortality. The estimates of observational error are negatively biased but usually not more than 10%. The estimates of the underlying variability of recruitment are positively biased but again usually not more than 10%.

It is useful to compare our results with those obtained from a VPA of the same populations (Fig. 4). Myers et al. (1990) estimated the variance and autocorrelation in recruitment for a large number of populations. We use their analysis when possible. For the New England populations in which a VPA was not used in Myers et al. (1990), we used the VPA from the latest assessment. We compare the standard deviation of log recruitment estimated at the earliest age of the research survey used in the analysis with that estimated from the VPA. In general the estimated standard deviation in log recruitment is larger in our analysis. This is consistent with density-dependent mortality, i.e., the variability in recruitment at the earlier ages is attenuated. Aging errors in the VPA also reduce the estimation variability in recruitment and induce autocorrelations. The autocorrelation estimated from the research surveys is usually less than that estimated by VPA (Fig. 4).

The degree of density-dependent mortality is so strong that the variation in abundance can be much less for older ages. For example, λ is estimated to be 0.41 for the age 1 whiting in October for the Irish Sea Surveys (Table 3). This would imply that a 10-fold increase in abundance during the first survey, at age 0, would result in only a 2.6-fold increase a year later. The presence of such strong density-dependent mortality within cohorts may explain why there is often little relationship between stock and recruitment, i.e., large year classes that occur when population size is large may suffer higher mortality during the juvenile stage.

The pattern of negative autocorrelation in cod is consistent with density-dependent mortality between adjacent year classes. This may also result if there is a negative autocorrelation between environmental factors causing recruitment variation. Myers et al. (1990) found that estimates of recruitment from VPA's were usually positively autocorrelated. Walters (1990) suggested that the autocorrelation in the residuals from a stock recruitment function was generally between 0.2 and 0.6. The autocorrelation in recruitment that we observed is less; our median autocorrelation was 0.15. Simulation studies indicate that the relative biases of our estimates of the autocorrelation are small (Appendix 2). This level of autocorrelation should be greater than that observed in the residuals from a fit of a stock and recruitment function because some of the autocorrelation we calculated is caused by both autocorrelated population size and any functional relationship between stock and recruitment.

Our model assumes that density-dependent mortality is proportional to the log of the initial juvenile density. This is likely to be true only over a portion of the range of juvenile densities. At low juvenile densities, we would expect very little density-dependent mortality. This may be the reason that very over-exploited populations, e.g., most of the New England populations, show relatively little evidence of density-dependent mortality.

There is correlation between estimation errors in surveys of different cohorts taken in the same year of the same survey. This implies that there are strong year effects in the surveys and that the standard errors calculated from survey design models underestimate the true estimation errors. The methods described in this paper allow the true estimation errors of the surveys to be estimated by treating different surveys as replicates. Our study directly estimates the estimation error variance of surveys. These estimates can be used to assess the reliablity of survey estimates when estimating population abundance (Rosenberg et al. 1992).

This study has clearly demonstrated the importance of density-dependent mortality in the juveniles. We do not understand why it is more important in some species, e.g. cod, and less important in others, e.g. haddock. Research studies of density-dependent habitat utilization of juveniles may clarify this difference.

Acknowledgements

We thank Jim Baird, Bjarte Bogstad, M. Bradford, Keith Brander, Ray Conser, Wendy W. Gabriel, M. Hilden, Tore Jakobsen, Andy Rosenberg, C. Walters, and W. Warren for assistance in understanding the data sets and methods. We thank the Northern Cod Science Program for financial assistance.

References

ANONYMOUS. 1991. Report of the Twelfth Northeast Regional Stock Assessment Workshop. Northeast Fish. Sci. Ref. Doc. 91-03, Woods hole, MA 02543, USA.

BAIRD, J.W., C.A. BISHOP, AND E.F. MURPHY. 1991. An assessment of the cod stock in NAFO Div. 3NO. NAFO SCR Doc. 91/83. Ser. No. N1967.

BEVERTON, R.J.H., AND T.C. ILES. 1992a. Mortality rates of O-group plaice (*Pleuronectes platessa* L.), dab (*Limanda limanda* L.) and turbot (*Scophthalmus maximus* L.) in European waters. II. Comparison of mortality rates and construction of life-table for O-group plaice. Neth. J. Sea Res. 29: 49–59.

BEVERTON, R.J.H., AND T.C. ILES. 1992b. Mortality rates of O-group plaice (*Pleuronectes platessa* L.), dab (*Limanda limanda* L.) and turbot (*Scophthalmus maximus* L.) in European waters. III. Density-dependence of mortality rate in O-group plaice, and some demographic implications. Neth. J. Sea Res. 29: 61–79.

BOLLEN, K.A. 1989. Structural equations with latent variables. Wiley, New York, N.Y. 541 p.

BRADFORD, M.J. 1991. Effects of aging errors on recruitment time series estimated from sequential population analysis. Can. J. Fish. Aquat. Sci. 48: 555–558.

BRANDER, K., AND S. SYMONDS. 1981. Gadoid recruitment surveys, Irish Sea 1979–1981. Ann. Biol. 38: 256–260.

CONSER, R., W. OVERHOLTZ, AND L. O'BRIEN. 1992. Assessment of yellowtail flounder. Working Pap. No. 12 from the 12th SAW Workshop, 1992, Woods Hole, MA 02543, USA.

HILBORN, R., AND C.J. WALTERS. 1992. Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Chapman and Hall, New York, N.Y. 570 p.

ICES. 1991a. Report of the roundfish working group. ICES Doc. C.M. 1991/Assess:4.

ICES. 1991b. Report of the North Sea flatfish working group. ICES Doc. C.M.

1991/Assess:5.
ICES. 1991c. Report of the Irish Sea and Bristol Channel working group. ICES Doc. C.M. 1991/Assess:1.
ICES. 1991d. Report of the Arctic fisheries working group. ICES Doc. C.M. 1991/Assess:3.
ILES, T.C., AND R.J.H. BEVERTON. 1991. Mortality rates of O-group (*Pleuronectes platessa* L.), dab (*Limanda limanda* L.) and turbot (*Scophthalmus maximus* L.) in European Waters. Neth. J. Sea Res. 27(3/4): 217–235.
KUZMIN, S.A. 1991. Assessment of the cod stock in NAFO Subarea 3 by the data from the 1990 trawl-acoustic survey. NAFO SCR Doc. 91/5. Ser. No. N1877: 19 p.
LOCKWOOD, S.J. 1980. Density-dependent mortality in O-group plaice (*Pleuronectes platessa* L.) populations. J. Cons. Int. Explor. Mer. 39: 148–153.
MYERS, R.A. 1991. Recruitment variability and range of three fish species. NAFO Sci. Counc. Stud. 16: 21–24.
MYERS, R.A., W. BLANCHARD, AND K.R. THOMPSON. 1990. Summary of North Atlantic fish recruitment 1942–1987. Can Tech. Rep. Fish. Aquat. Sci. 1743: 108 p.
MYERS, R.A., AND N.G. CADIGAN. 1993. Is juvenile natural mortality in marine demersal fish variable? Can. J. Fish. Aquat. Sci. 50: 1591–1598.
MYERS, R.A., AND K. DRINKWATER. 1989. The influence of Gulf Stream warm core rings on recruitment of fish in the Northwest Atlantic. J. Mar. Res. 47: 635–656.
MYERS, R.A., AND K. STOKES. 1989. Density-dependent habitat utilization of groundfish and the improvement of research surveys. ICES C.M. 1989/D:15.
POPE, J.G. 1972. An investigation of the accuracy of virtual population analysis. ICNAF Res. Bull. 9: 65–74.
RAUCK, G., AND J.J. ZIJLSTRA. 1978. On the nursery aspects of the Wadden Sea for some commercial fish species and possible long-term changes. Rapp. P.-V. Reun Cons. Int. Explor. Mer 172: 266–275.
RICKER, W.E., AND R.E. FOERSTER. 1948. Computation of fish production. Bull. Bingham Oceanogr. Collect. Yale Univ. 11: 173–211.
ROSENBERG, A.A., G.P. KIRKWOOD, R.M. COOK, AND R.A. MYERS. 1992. Combining information from commercial catches and research surveys to estimate recruitment: a comparison of methods. ICES J. Mar. Sci. 49: 379–387.
SHEPHERD, J.G., AND D.H. CUSHING. 1980. A mechanism for density-dependent survival of larval fish as the basis of a stock–recruitment relationship. J. Cons. Int. Explor. Mer 39: 160–167.
SHEPHERD, J.G., AND D.H. CUSHING. 1990. Regulation in fish populations: myth or mirage? Philos. Trans. R. Soc. Lond. B 330: 151–164.
SUNDBY, S., H. BJORKE, A.V. SOLDAL, AND S. OLSEN. 1989. Mortality rates during the early life stages and yearclass strength of north east Arctic cod (*Gadus morhua* L.). Rapp. P.-V. Reun. Cons. Perm. Int. Explor. Mer 191: 351–358.
URSIN, E. 1982. Stability and variability in the marine ecosystem. Dana 2: 51–67.
VEER, H.W. VAN DER. 1986. Immigration, settlement and density-dependent mortality of a larval and early post-larval O-group (*Pleuronectes platessa*) population in the western Wadden Sea. Mar. Ecol. Prog. Ser. 29: 223–236.
VEER, H.W. VAN DER, L. PIHL, AND M.J.N. BERGMAN. 1990. Recruitment mechanisms in North Sea plaice (*Pleuronectes platessa*). Mar. Ecol. Prog. Ser. 64: 1–12.
WALTERS, C.J. 1990. A partial bias correction factor stock–recruitment parameter estimation in the presence of autocorrelated environmental effects. Can. J. Fish. Aquat. Sci. 47: 516–519.

Appendix 1. Model Estimation

Recall that the model considered is

$$l_{t,a,i} = c_{a,i} - m_a + \lambda_{a,i} L_{t,0} + \delta_{t,a,i}$$

$$L_{t,0} = L_{t,i} + \beta L_{t-1,0}.$$

The parameters $c_{a,i} - m_a$ are removed by standardizing each $l_{t,a,i}$ (subtracting the sample mean, $\frac{1}{T}\sum_{t=1}^{T} l_{t,a,i}$, where T is the number of years in the research survey). Hence, $l_{t,a,i}$, $L_{t,0}$, and $L_{t-1,0}$ are all in terms of deviations from their means.

To illustrate the subscripts used in the model, consider the data in Table 1. Here, $n_{t,0,1}$, $n_{t,1,1}$, and $n_{t,2,1}$ denote recruitment estimates of cohort t from estimates of 0, 1, and 2-yr-olds in years t, $t + 1$, and $t + 2$, respectively, from the EGFS. For example, $n_{1979,0,1} = 18.60$, $n_{1979,1,1} = 50.80$, $n_{1979,2,1} = 16.90$, etc. The a subscript denotes the age of fish (in years); it is a truncation of the actual age of the fish (in fraction years). Hence, the fish $l_{t,1,2}$ and $l_{t,1,1}$ are not necessarily of the same age. The actual ages are shown in Fig. 2 and 3. For this reason, we estimate separate λ's for different surveys unless they actually are of the same age. The surveys are listed in order by age in Tables 2–5.

The above model is written in terms of realizations of random variables (observations), unknown parameters, and latent random variables. This suggests that a latent variable (i.e., confirmatory factor) analysis is appropriate. Using the assumptions presented in the Methods section, it is possible to estimate the above parameters using maximum likelihood methods. We write the model for the data in Table 1 in matrix form and translate our notation with common notation (LISREL, Bollen 1989) in the statistical literature.

Let $\boldsymbol{l}_t$ be a vector of standardized log recruitment estimates at time t and $t - 1$:

$$\boldsymbol{l}'_t = [l_{t,0,1}, l_{t,1,2}, l_{t,1,1}, l_{t,2,2}, l_{t,2,1}, l_{t-1,0,1}, l_{t-1,1,2}, l_{t-1,1,1}, l_{t-1,2,2}, l_{t-1,2,1}]$$

$$t = 2, 3, \ldots, T.$$

Also, let $\boldsymbol{L}_t$ be a latent vector variable of standardized log recruitment at time t and $t - 1$:

$$\boldsymbol{L}_t = \begin{bmatrix} L_{t,0} \\ L_{t-1,0} \end{bmatrix}.$$

Construct a matrix of λ's as follows:

$$\boldsymbol{\Lambda} = \begin{bmatrix} 1 & 0 \\ \lambda_{1,2} & 0 \\ \lambda_{1,1} & 0 \\ \lambda_{2,2} & 0 \\ \lambda_{2,1} & 0 \\ 0 & 1 \\ 0 & \lambda_{1,2} \\ 0 & \lambda_{1,1} \\ 0 & \lambda_{2,2} \\ 0 & \lambda_{2,1} \end{bmatrix}.$$

Let $\boldsymbol{\delta}_t$ be a vector variable of observational errors at time t and $t - 1$:

$$[\delta_{t,0,1}, \delta_{t,1,2}, \delta_{t,2,2}, \delta_{t,2,1}, \delta_{t-1,0,1}, \delta_{t-1,1,2}, \delta_{t-1,1,1}, \delta_{t-1,2,2}, \delta_{t-1,2,1}].$$

In matrix notation, the model may be written as

$$\boldsymbol{l}_t = \boldsymbol{\Lambda}\, \boldsymbol{L}_t + \boldsymbol{\delta}_t.$$

The distributional assumptions presented in the Methods section are $\boldsymbol{L} \sim N(\boldsymbol{0}, \boldsymbol{\Phi})$, $\boldsymbol{\delta}_t \sim N(\boldsymbol{0}, \boldsymbol{\Theta})$, and $E(\boldsymbol{L}_t \boldsymbol{\delta}'_t) = \boldsymbol{0}$. $\boldsymbol{\Phi}$

and Θ are

$$\Phi = \begin{bmatrix} \phi_{1,1} & \phi_{1,2} \\ \phi_{2,1} & \phi_{2,2} \end{bmatrix}$$

and for this example

$$\Theta = \begin{bmatrix} \theta_{0,1} & 0 & 0 & 0 & 0 & 0 & 0 & \theta_{0,1}^{1,1} & 0 & 0 \\ & \theta_{1,2} & 0 & 0 & 0 & 0 & 0 & 0 & \theta_{1,2}^{2,2} & 0 \\ & & \theta_{1,1} & 0 & 0 & \theta_{0,1}^{1,1} & 0 & 0 & 0 & \theta_{1,1}^{2,1} \\ & & & \theta_{2,2} & 0 & 0 & \theta_{1,2}^{2,2} & 0 & 0 & 0 \\ & & & & \theta_{2,1} & 0 & 0 & \theta_{1,1}^{2,1} & 0 & 0 \\ & & & & & \theta_{0,1} & 0 & 0 & 0 & 0 \\ & & & & & & \theta_{1,2} & 0 & 0 & 0 \\ & & & & & & & \theta_{1,1} & 0 & 0 \\ & & & & & & & & \theta_{2,2} & 0 \\ & & & & & & & & & \theta_{2,1} \end{bmatrix}.$$

We constrain $\phi_{1,1} = \phi_{2,2} = \phi$. The β parameter is estimated as $\beta = \frac{\phi_{2,1}}{\phi}$. The notation $\theta_{i,j}^{k,l}$ denotes the covariance between $\delta_{i,j}$ and $\delta_{k,l}$.

In the statistical literature (e.g., Bollen 1989), $\boldsymbol{L}_t$ would commonly be denoted as $\boldsymbol{x}_t$ and $\boldsymbol{I}_t$ as $\boldsymbol{L}_t$. Our notation (except subscripts and superscripts) is otherwise standard.

The theoretical covariance matrix derived from this model is

$$\Sigma = \Lambda \Phi \Lambda' + \Theta$$

This is a 10×10 matrix with 14 parameters to estimate. The sample covariance matrix $\boldsymbol{S}\left(\frac{1}{T}\Sigma_{t=1}^{T}\, l_t l_t'\right)$ is also a 10×10 matrix with 55 unique terms.

The basic estimation procedure is to fit the observed variance–covariance matrix with the theoretical one. The fitting function that is minimized in order to find the maximum likelihood estimates is

$$\log|\Sigma| + \mathrm{tr}(\boldsymbol{S}\Sigma^{-1}) - \log(|\boldsymbol{S}|) - p$$

where tr is the matrix trace and p is the dimension of $\boldsymbol{S}$. The models are estimated using SAS's PROC CALIS with the pairwise covariance matrix. If this matrix is not positive definite, the ridge option in PROC CALIS is used to perturb the matrix so that it is positive definite.

Maximum likelihood estimates do not always produce sensible results, i.e., negative θ's are estimated, usually because the data series are relatively short. Any model who's estimation yielded negative θ's is rejected. for this reason, the North Sea cod model is estimated only from data containing no missing values. Also, one θ in the North Sea sole and Northeast Arctic haddock data is constrained to have a small positive variance.

The unconstrained λ's are multivariate normal in distribution (an asymptotic result from maximum likelihood theory). The mean of this distribution is estimated from the parameter estimates. The covariance matrix is estimated by the information matrix evaluated at the parameter estimates. As a test of whether the λ's are <1, the joint p-values that all λ's are <1 are presented. The p-values are approximated using 10 000 Monte Carlo samples. This sampling procedure produced two-digit accuracy when applied to some known results. The p-values presented for $\rho(\cdot)$ in Tables 2–5 are based upon a normal approximation of the estimated covariance and not $\rho(\cdot)$ because of difficulties in obtaining standard errors for derived parameters.

An issue that must be addressed is model identification. Note that it is not necessary to understand what follows to understand the data analysis. A parameter in Σ is identified if it can be expressed as a function of one or more elements in S. A parameter is underidentified if it cannot be expressed only as a function of some elements in $\boldsymbol{S}$ (Bollen 1989). Let $\Sigma_{\dot{\omega}}$ be the covariance matrix constructed from a particular set, $\dot{\omega}$, of free parameters in the model. If a model is identified, then for $\dot{\omega}$, and another set, $\ddot{\omega}$,

$$\Sigma_{\dot{\omega}} = \Sigma_{\ddot{\omega}} \Rightarrow \dot{\omega} = \ddot{\omega}.$$

The theoretical covariance matrix of all models considered in this paper may be represented as follows:

$$\Sigma_{\dot{\omega}} = \dot{\Lambda}\, \dot{\Phi}\, \dot{\Lambda}' + \dot{\Theta}$$

where

$$\dot{\Lambda} = \begin{bmatrix} \dot{\lambda} & \mathbf{0} \\ \mathbf{0} & \dot{\lambda} \end{bmatrix}, \lambda' = (1, \dot{\lambda}_1, \ldots, \dot{\lambda}_{l-1}),$$

$$\mathbf{0} = l \times 1 \text{ vector of zero's},$$

$$\dot{\Phi} = \begin{bmatrix} \dot{\phi}_{1,1} & \dot{\phi}_{1,2} \\ \dot{\phi}_{1,2} & \dot{\phi}_{1,1} \end{bmatrix},$$

$$\dot{\Theta} = \begin{bmatrix} \dot{\Theta}_{1,1} & \dot{\Theta}_{1,2} \\ \dot{\Theta}_{1,2} & \dot{\Theta}_{1,1} \end{bmatrix}, \dot{\Theta}_{1,1} = \mathrm{Diag}\,\dot{\theta}_{1,1}, \ldots, \dot{\theta}_{l,l}),$$

$$\dot{\Theta}_{1,2} = l \times l \text{ symmetric matrix}.$$

The t-rule (Bollen 1989) always holds for this model when $l > 1$. This is a necessary but not sufficient condition for the model to be identified. Our model does not fall within any of the classes of models in Bollen (1989) for which sufficient conditions for identification have been constructed. The sufficient condition is that one (i, j) pair in $\Theta_{1,2}$ be fixed. This follows by observing that

$$\Sigma_{\dot{\omega}} = \Sigma_{\ddot{\omega}} \Rightarrow$$

$$\dot{\phi}_{1,1}\dot{\lambda}\dot{\lambda}' + \dot{\Theta}_{1,1} = \ddot{\phi}_{1,1}\ddot{\lambda}\ddot{\lambda} + \ddot{\Theta}_{1,1}$$

$$\dot{\phi}_{1,2}\dot{\lambda}\dot{\lambda}' + \dot{\Theta}_{1,2} = \ddot{\phi}_{1,2}\ddot{\lambda}\ddot{\lambda} + \ddot{\Theta}_{1,2}.$$

From the first equation, it is easy to show that $\dot{\phi}_{1,1} = \ddot{\phi}_{1,1}$, $\dot{\lambda} = \ddot{\lambda}$, and $\dot{\Theta}_{1,1} = \ddot{\Theta}_{1,1}$. If $\dot{\lambda}$ is substituted for in the second equation above, and one (i, j) pair in $\dot{\Theta}_{1,2}$ is fixed, then $\dot{\phi}_{1,2} = \ddot{\phi}_{1,2}$ and hence, $\dot{\Theta}_{1,2} = \ddot{\Theta}_{1,2}$. With this constraint, we now have that $\Sigma_{\dot{\omega}} = \Sigma_{\ddot{\omega}} \Rightarrow \dot{\omega} = \ddot{\omega}$, that is, the model is identified. For the populations considered here, most of the elements in $\Theta_{1,2}$ are fixed as zero's.

TABLE A2.1. Simulation results to test the robustness of the estimation model. The number of years of data used to estimate the model is n, $D(\cdot)$ denotes distribution of $(\cdot)$; L, lognormal; G, gamma; LG, loggamma. See text for details.

No.	n	$D(\xi)$	$D(\delta_i)$	θ/ϕ	$\theta_{1,1}/\theta$	Mean bias $\lambda_2 - \lambda_4$	Mean bias $\theta_1 - \theta_4$	Bias ϕ	% simulations converged with positive variances
1	100	L	L	1	1	0.013	−0.020	0.010	
2	100	L	L	0.2	1	0.005	−0.002	−0.002	100
3	20	L	L	1	1	0.186	−0.080	0.084	90.1
4	20	L	L	0.2	1	0.013	−0.014	0.002	98.3
5	10	L	L	1	1	0.137	−0.129	0.313	59.2
6	10	L	L	0.2	1	0.022	−0.021	0.078	79.4
7	20	G	L	1	1	0.196	−0.088	−0.131	82.8
8	20	G	L	0.2	1	0.013	−0.012	−0.185	98.3
9	20	L	LG[a]	1	1	0.145	−0.076	0.087	88.9
10	20	L	LG[a]	0.2	1	0.017	−0.011	0.008	87.5
11	20	L	LG[b]	1	1	0.128	−0.079	0.104	87.3
12	20	L	LG[b]	0.2	1	0.011	−0.010	0.027	86.2
13	20	L	L[c]	1	1	0.142	−0.069	0.118	90.3
14	20	L	L[c]	0.2	1	0.015	−0.008	0.025	98.2
15	20	L	L	1	4	0.216	−0.127	0.540	76.9
16	20	L	L	0.2	4	0.064	−0.019	0.042	95.3
17	20	L	L	1	9	−0.141	−0.250	0.979	72.0
18	20	L	L	0.2	9	0.123	−0.036	0.144	93.8

[a]Left-skewed.
[b]Right-skewed.
[c]Correlated errors.

Appendix 2. Robustness

We examined three questions about the reliability of our results: (1) Could our results be an artifact of the sampling gear? (2) Are our results robust to violations of the model assumptions? (3) What are the small sample biases of our estimators?

First, it is possible that the catchability of the research gear is density dependent, i.e., that the efficiency of the trawl increases for small fish when the net becomes clogged. This is unlikely because the research surveys use mesh small enough to catch all or nearly all fish of the size considered here. However, catchability may still increase with age because catchability also depends upon behavior, e.g., smaller fish may have a different vertical distribution.

The second question is partially addressed by relaxing the parametric assumptions for the statistical distribution describing recruitment and the form of the estimation errors. The maximum likelihood estimation procedure used requires log recruitment and the estimation errors to be normally distributed. Although this is a reasonable assumption (Myers et al. 1990), it is possible to use estimation methods for which this assumption is not required. In particular, our results are tested by using weighted and unweighed least squares estimation. To save space the results are not presented, but for weighted least squares the basic pattern in the results is usually very similar. Although the unweighed least squares solution is not generally scale invariant and is not generally comparable with maximum likelihod or weighted least squares (Bollen 1989), it is in our case because the logarithmic transformation removes the effects of a scalar transformation of the sample covariance matrix. This solution again produced the same general results although the parameter estimates are sometimes of different magnitude.

Simulations are conducted to investigate the small sample properties of the estimators used and further test violations of the model assumptions. The model assuming independence between adjacent cohorts is used (Eq. 1). The general simulation procedure is to (1) fix the sample size (n) to be investigated, (2) fix the distribution of the model latent recruitment ($L_{t,0}$) and the errors ($\delta_{t,a,i}$), (3) specify the parameters, (4) generate 1000 data sets each with n random observations of $L_{t,0}$ and $\delta_{t,a,i}$, (5) use these values to generate the standardized survey data, and (6) estimate parameters using the standard distributional assumptions given in the Methods section.

Our interest is to determine if statistical artifacts could cause the estimated λ's to be less than 1; therefore, all λ's are set at 1 to investigate this in the simulations. The error variances (θ) are equal to and one fifth of the variance of ϕ which is set as 1. These ratios of variances are rough bounds on estimates in Tables 2–5.

In the first six simulations the effect of sample size on the accuracy of parameter estimates at two levels of ϕ/θ (Table A2.1) is investigated. $L_{t,0}$ and δ are generated as standard normal random variables (rv's) with zero means and variances equal to 1 and θ, respectively. These normality assumptions are the same as used in the data analysis.

Simulation's 7 through 14 are conducted to investigate the robustness of the estimators to violations in distributional assumptions. In simulations 7 and 8 the distribution of $L_{t,0}$ over time was generated as a gamma rv with a mean and variance constrained to equal that of the log of a standard normal rv (i.e., $\phi = 1$). Left-skewed errors (δ's) were generated from a gamma rv with shape and scale parameters 2 and $1/\sqrt{2}$, respectively; these are considered as reasonable parameters. A right-skewed gamma deviate is generated as the negative of a left-skewed deviate. In simulations 9–12, these rv's are centered to have mean 0 (they are error terms). In simulations 13 and 14, correlated errors are produced by adding 0.1 to the first half of the time series of errors ($\delta_{1,1}$) and -0.1 to the later half. $\delta_{2,1}$ is modified by the same procedure except 0.1 is added to the first half + 1 of the errors. This is done to simulate a change in the efficiency of a survey half way through the survey series.

In the last four simulations, the effect of unequal estimation error variance is investigated. of particular concern is that larger estimation error variances at younger ages might lead to biased results for small sample sizes. We considered an estimation error variance of the younger age, $\theta_{1,t}$, equal to 4 or 9 times the variance of the other error terms, which are all equal (θ).

The analyses indicate that most of the potential biases in parameter estimates result from small sample sizes and not deviations in model assumptions. Generally the biases resulting from deviations in distributional assumptions do not differ substantially compared with those obtained (at the same sample size) when the distributional assumptions are exact. The simulations indicate that our estimates of density-dependent mortality are not generally artifacts of violations in model assumptions because the simulated biases are too small and are of the wrong sign, except if the estimation error variance is large.

If the estimation error variance for the youngest age is much larger than for older ages and the estimation error variances of the older ages are similar to ϕ, then very large biases may result. In particular, density-dependent mortality may be estimated to exist when there is none. The estimates of variance parameters in Tables 2–5 for most populations are similar to the parameters in simulations 3, 4, and 16 in Table A2.1. The biases here are not substantial, particularly for Nos. 4 and 16. Five populations have variance estimates somewhere between those considered in simulations 3 and 15. These populations are Irish Sea whiting, Gulf of Maine cod, Georges Bank yellowtail flounder, southern New England yellowtail flounder, and southern Grand Bank cod. While one might consider these biases as being substantial, they would not result in false conclusions. The variance estimates from all populations are not in the range of simulation 15; hence, we conclude that estimates of density-dependent mortality are not statistical artifacts.

A referee pointed out that there is a potential for the estimate of autocorrelation of recruitment, β, to be negatively biased. As part of ongoing research into the small sample properties of maximum likelihood estimates for latent variable models, further analyses (simulations) have been conducted in which the bias of the autocorrelation is investigated. The results are too bulky to report here in detail but for $n = 10$, it was found that when the ratio of the estimation error variance (θ) to the latent variable variance (ϕ) was small (0.1) the %bias in the estimate of the correlation was between −2 and 7% depending upon if the actual correlation was small (0.1) or large (0.9); when the θ/ϕ ratio was 1 the %bias ranged between 2 and 17%. These biases are small and not a cause for great concern. Although the model in the unreported simulations is essentially the same as the one in Appendix 1, more parameters were estimated; however, the fact that the correlation between latent variables (i.e., underlying recruitment) is estimated relatively accurately should still apply.

Is Juvenile Natural Mortality in Marine Demersal Fish Variable?

Ransom A. Myers and Noel G. Cadigan

Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada

Myers, R.A., and N.G. Cadigan. 1993. Is juvenile natural mortality in marine demersal fish variable? Can. J. Fish. Aquat. Sci. 50: 1591–1598.

We test the hypothesis that the interannual variability of the density-independent component of juvenile natural mortality is a major source of variability in abundance of marine demersal fish. Our tests use data on populations for which there are research surveys consisting of two simultaneous estimates of abundance of young juveniles soon after settlement and two more simultaneous estimates of the same cohort a year later. We applied our test to 14 populations of Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*), plaice (*Pleuronectes platessa*), and sole (*Solea vulgaris*). We conclude that, in all populations examined except North Sea sole, there was very little or no interannual variability in the density-independent component of juvenile mortality.

Nous vérifions l'hypothèse que la variabilité interannuelle de la composante indépendante de la densité de la mortalité naturelle chez les juvéniles est la principale cause de la variabilité de l'abondance des poissons démersaux. Pour nos vérifications nous utilisons des données sur les populations pour lesquelles il existe des relevés de recherche constitués de deux estimations simultanées de l'abondance des jeunes juvéniles, peu après la sédentarisation, et deux autres estimations simultanées de la même cohorte une année plus tard. Nous avons appliqué notre épreuve à 14 populations de morues franches (*Gadus morhua*), d'églefins (*Melanogrammus aeglefinus*), de merlans (*Merlangius merlangus*), de plies (*Pleuronectes platessa*) et de soles (*Solea vulgaris*). Nous concluons que, dans toutes les populations examinées, sauf la sole de la mer du Nord, il n'y avait presque pas de variabilité interannuelle de la composante de la mortalité juvénile qui est indépendante de la densité.

Received December 2, 1992
Accepted April 28, 1993
(JB715)

Reçu le 2 decembre 1992
Accepté le 28 avril 1993

The variability of year class strength is a fundamental problem in the population biology of marine fish. Hjort (1914, 1926) hypothesized that the principle life stage in which interannual variability in survival occurred was the larval planktonic stage. Recently, it has been suggested that the life stage in which interannual variability in survival is most important is the juvenile stage (May 1984; Smith 1985). Although there are many estimates of the interannual variability in recruitment (Myers et al. 1990), there have been very few estimates of the interannual variability in juvenile survival. This hypothesis is difficult to test because variable survival cannot usually be separated from errors in estimating abundance. Peterman et al. (1988) argued that the major source of variability in recruitment in the northern anchovy (*Engraulis mordax*) was variable survival between 19-d-old larvae until age 1. However, Peterman et al. (1988) had no estimates of the errors in estimating either quantity.

Here, we attack this problem by analyzing populations in which there are two simultaneous estimates of abundance during age 0 + and two more simultaneous estimates a year later. The independent, simultaneous surveys were not usually carried out as part of a designed research program, but because two nations had overlapping claims to the same fish population. If there are multiple estimates of abundance, it is possible to separate variable survival from errors in estimation. To fix ideas, consider a population in which there are two simultaneous surveys to estimate abundance at age 0 and two more a year later at age 1. Assume in this simple example only that the variance in estimating abundance is the same for all surveys. If there is no interannual variability in survival between ages 0 and 1, then the correlations between the survey estimates should be the same at the same age as between ages. However, if there is large interannual variability in survival between the ages then there should be higher correlations in the surveys at the same age than between ages. The analysis below is an elaboration of this simple idea to the general case in which the variance of the survey errors is unknown and unequal.

Our approach here is to (1) develop a statistical model of variable juvenile mortality, (2) compile a large number of data sets that would allow this hypothesis to be tested, (3) apply the model systematically to each data set, and (4) investigate the power of our statistical test.

We consider two components of interannual variability in survival: (1) the density-dependent component and (2) the density-independent component. In the companion paper to this one (Myers and Cadigan 1993), we used similar methods and data to examine density-dependent survival.

An Example: North Sea Sole

Before we develop a formal model, it is useful to consider an example. On the continental side of the North Sea, there are inshore and offshore surveys for juvenile flatfish (Table 1). The index used for the INSHORE surveys was weighted by area and corrected for differences in efficiency between the survey gear. The units for the TRIDENS surveys are the number of fish caught per 100 h of fishing.

The correlation between the age 0 surveys is high ($r = 0.81$, $p < 0.001$) (Fig. 1). Similarly the correlations between the age 1

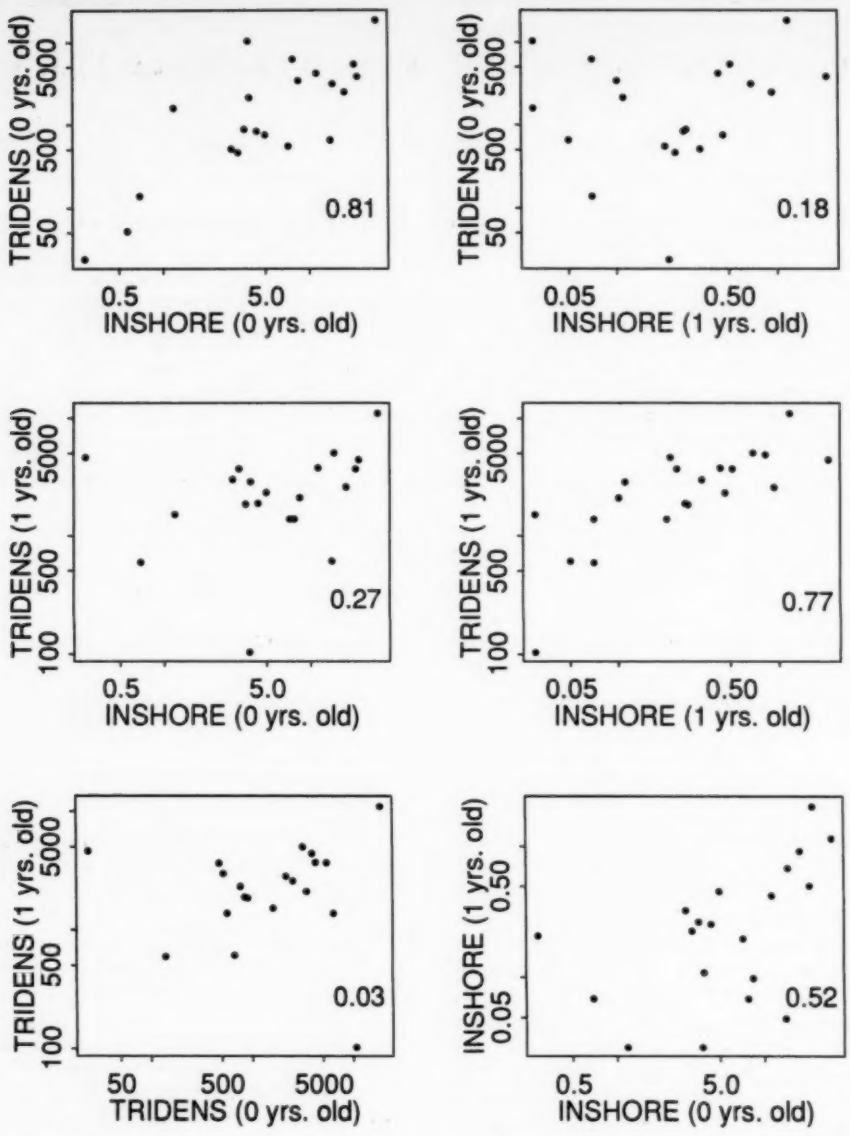


FIG. 1. Pairwise plots of abundance estimates for North Sea sole (Table 1). The estimates are log transformed. The correlation coefficient is presented in the lower right corner.

surveys is high ($r = 0.77$, $p < 0.001$); however, the correlation between the surveys at different ages is much lower, ranging from 0.03 to 0.52. One explanation for this result is that both surveys are reasonable estimates of abundance but that variable survival takes place during the year between surveys. There is also evidence that density-dependent survival is a linear function of the abundance (Fig. 2). Abundance is taken as the log numbers at age 1 and survival is taken as the difference in log numbers at ages 2 and 0. Note that the 1972 year class is very low in both 0+ surveys but appears to be close to normal in the 1+ surveys. Thus, we include density-dependent survival in the model in a manner similar to Myers and Cadigan (1993).

The Model

Consider a population in which each cohort is surveyed simultaneously at two different ages. Let the number of fish in cohort t surveyed at age a be $N_{t,a}$. The surveys see the juveniles at two ages; the survey index of the youngest age is 0 and surveys that see the fish at later ages are labeled 1. The natural mortality is divided into three components: (1) the average mortality between the two ages that is independent of density, m, (2) the density-dependent component which is assumed to be proportional to the logarithm of the initial density, $(1 - \lambda) \log N_{t,0}$, and (3) the variable component unrelated to abundance, ε_t. It is

TABLE 1. Data for the North Sea sole population from the TRIDENS (in units of numbers per 100 h of fishing) and INSHORE surveys (in units of numbers per 1000 m^2).

Year class	TRIDENS 0-yr-olds	TRIDENS 1-yr-olds	INSHORE 0-yr-olds	INSHORE 1-yr-olds
1969		4 938		0.82
1970	669	613	13.88	0.05
1971	6 327	1 410	7.72	0.07
1972	24	4 686	0.29	0.21
1973	847	1 924	4.35	0.26
1974	140	597	0.69	0.07
1975	565	1 413	7.14	0.20
1976	475	3 724	3.23	0.23
1977	1 620	1 552	1.18	0.03
1978	10 529	104	3.80	0.03
1979	3 908	4 483	21.24	2.03
1980	5 518	3 739	20.17	0.51
1981	3 194	5 098	14.40	0.69
1982	2 528	2 640	17.36	0.93
1983	769	2 359	4.96	0.46
1984	3 473	2 151	8.39	0.10
1985	4 268	3 791	11.19	0.43
1986	901	1 890	3.58	0.27
1987	18 690	11 227	28.66	1.16
1988	523	3 052	2.93	0.33
1989	2 171	2 900	3.89	0.11
1990	53		0.57	

mathematically convenient to estimate the density-dependent survival rate, λ, rather than mortality. The population dynamics is described by

$$(1)\quad N_{t,1} = N_{t,0}\, e^{-m-(1-\lambda)\log N_{t,0}+\varepsilon_t}.$$

Note that we assumed in Eq. 1 that recruitment was independent between adjacent cohorts. In Myers and Cadigan (1993), we examined autocorrelated recruitment and found that there was little effect on the estimation of the other parameters if the autocorrelation was assumed to be zero.

It is useful to work with log-transformed variables, e.g. $L_{t,a} = \log N_{t,a}$. Thus, (1) is written as

$$(2)\quad L_{t,1} = -m + \lambda L_{t,0} + \varepsilon_t.$$

The observed log-transformed data from survey i at age a is $l_{t,a,i}$. We assume that the estimates of abundance at age in each survey are proportional to the true abundance at that age. Let $c_{a,i}$ be the log catchability of survey i at age a, and let $\delta_{t,a,i}$ be the observation error of survey i at age a from cohort t. The observed log-transformed abundance from survey i is

$$(3)\quad l_{t,a,i} = c_{a,i} + L_{t,a} + \delta_{t,a,i}$$

Estimation

We are primarily interested in determining the interannual variability in juvenile survival. In order to estimate the parameters of the above model, we make the following assumptions:

(1) Log-transformed standardized recruitments are independent random variables from a normal distribution with constant mean and variance (i.e., independent of year and also (2) and (3) below). That is:

$$L_{t,0} \sim N(\mu, \phi)$$

(2) The variable components of mortality are independent random variables from a normal distribution with zero mean and constant variance (i.e., independent of year, (1) above, and (3) below). That is:

$$\varepsilon_t \sim N(0, \psi)$$

(3) Errors in estimating abundance of each age group from each research survey are independent random variables from a normal distribution with zero mean and constant variance (i.e., independent of year and also (1) and (2) above). That is:

$$\delta_{t,a,i} \sim N(0, \theta_{a,i}).$$

$$\begin{bmatrix} \mathrm{VAR}(l_{t,0,1}) & \mathrm{COV}(l_{t,0,1}, l_{t,0,2}) & \mathrm{COV}(l_{t,0,1}, l_{t,1,1}) & \mathrm{COV}(l_{t,0,1}, l_{t,1,2}) \\ & \mathrm{VAR}(l_{t,0,2}) & \mathrm{COV}(l_{t,0,2}, l_{t,1,1}) & \mathrm{COV}(l_{t,0,2}, l_{t,1,2}) \\ & & \mathrm{VAR}(l_{t,1,1}) & \mathrm{COV}(l_{t,1,1}, l_{t,1,2}) \\ & & & \mathrm{VAR}(l_{t,1,2}) \end{bmatrix}$$

$$= \begin{bmatrix} \phi + \theta_{0,1} & \phi & \lambda\phi & \lambda\phi \\ & \phi + \theta_{0,2} & \lambda\phi & \lambda\phi \\ & & \lambda^2\phi + \psi + \theta_{1,1} & \lambda^2\phi + \psi \\ & & & \lambda^2\phi + \psi + \theta_{1,2} \end{bmatrix}.$$

Note that the above matrix has seven unknown parameters to estimate and there are 10 terms in the upper triangular portion of the matrix. Also note that we do not attempt to estimate m, or the $c_{a,i}$. These parameters cannot be estimated without independent data or by making unreasonable assumptions. We estimate the other parameters in Σ by matching the observed variance covariance matrix (S) as close as possible. By inspection, we see that each model parameter can be uniquely written as a function of one or more elements in S; that is, the model is identified. We use maximum likelihood methods to obtain the estimates. Details of the estimation methods are described in Bollen (1989).

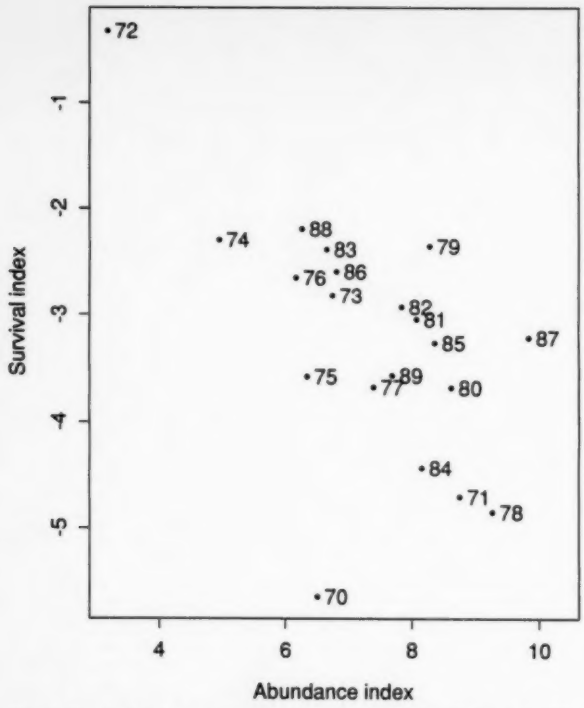


FIG. 2. Survival index versus abundance index for North Sea sole. The index of survival is the difference between the log numbers at age 0 and 1 from the INSHORE survey. The index of abundance is the log numbers at age 1 from the TRIDENS survey. Note that the estimation errors in the two surveys are independent.

With the above assumptions, it is possible to calculate the variance of each survey at age and the covariance between surveys. The theoretical variance–covariance matrix, Σ, is

Data

We attempted to compile and analyze all data sets in which there were two reliable, simultaneous research survey estimates of juvenile fish abundance. We had several criteria before a data set could be included in our analysis. First, the data series had to have estimates for at least 10 years. Second, we attempted to use surveys that covered the complete range of the population. Unfortunately, this was not always possible. Some surveys covered regions close to shore, while others, such as the North Wales Groundfish Surveys (NWGS), covered only a portion of the region. We have identified such limitations of the data below. Third, we had to deal with the problem of zero catches in some years. We attempted to use data series that only had positive catches. In several cases, we replaced zeros with one tenth the minimum estimate for that age. Fourth, the data sets had to be simultaneous or nearly simultaneous. Most of the surveys took place within a month of each other. The major exception is the southern Baltic survey for cod. We have used the data sets described in Myers and Cadigan (1993) when possible; however, the requirement that the surveys be simultaneous has led us to modify some of the data sets used. The sources of the data sets can be found in Myers and Cadigan (1993) unless otherwise stated.

The North Sea gadoid populations (Table 2) are estimated from three autumn surveys: the English Groundfish Surveys (EGFS) cover the whole North Sea, the Scottish Groundfish Surveys (SGFS) have better coverage in the north, and the Dutch Groundfish Survey (DGFS) have better coverage in the south. The EGFS has taken place since 1977, the DGFS since 1980, and the SGFS since 1982. Atlantic cod (*Gadus morhua*), which has its maximum abundance in the southern part of the North Sea, is well estimated by the EGFS and DGFS, while haddock (*Melanogrammus aeglefinus*), which has its maximum abundance in the north, is best estimated by EGFS and SGFS. Whiting (*Merlangius merlangus*) is covered by all three surveys. We use the EGFS and DGFS for this population because they are the longest.

The eastern North Sea populations of sole (*Solea vulgaris*) and plaice (*Pleuronectes platessa*) (Table 2) were surveyed during the autumn (September–October) from the Dutch to the Danish coastal waters using the research vessel *Tridens*. There are surveys carried at approximately the same time in more inshore regions during the same time period from the Belgium to the Danish coastal waters. These are autumn surveys (September–October) that are primarily aimed at estimating the numbers of 0+ recruits.

The Irish Sea gadoid populations (Table 3) are estimated from the October NWGS and the autumn (September–October) prerecruit gadoid surveys, denoted as GADOID. The Irish Sea plaice population is surveyed by the NWGS and the Irish September survey. In the Irish Sea prerecruit gadoid survey, there were two years when no age 0+ cod were caught. In these years the zeros were replaced by one tenth the lowest catch in the series for 0+ cod.

Random stratified surveys of the Gulf of Maine (Table 4) and Georges Bank cod populations have taken place in the offshore regions in the spring (since 1968) and the fall (since 1963) and in the inshore regions by the State of Massachusetts in the spring (since 1978) and autumn (since 1978). There was no age in which cod from the Georges Bank population was caught effectively in both surveys, so only the analysis for the Gulf of Maine population is given. We use the fall 0+ and spring 1+ surveys in the analysis. There was no other species in the region that was reliably estimated simultaneously from both surveys.

Surveys of cod (Table 4) on the southern Grand Banks of Newfoundland have taken place since 1972 by Canada and since 1978 by the USSR. Both surveys are in the spring and cover the same region. Random stratified surveys of cod (Table 4) on the St. Pierre Bank south of Newfoundland have taken place since 1972 by Canada (Bishop et al. 1991) and since 1978 by France (Moguedet 1991). Both surveys are in the spring and cover the same region with the exception that the Canadian surveys extend into deeper water.

The Northeast Arctic haddock population (Table 5) has been surveyed in the Barents Sea by the USSR since 1948 during late autumn–early winter (November–December) using trawl surveys and since 1977 during winter (January–March) by Norway using acoustic surveys. We examined survival for one year from the 0+/early 1+ stage. The Northeast Arctic cod population does not seem to be as well surveyed by the acoustic surveys; there are several years in which no age 1+ cod were caught. We therefore use the bottom trawl survey for cod carried out from January to March in the Barents Sea since 1983 which did not record zero catches for any age class. For the USSR trawl surveys for cod, we use the combined index for ICES subareas I, IIa, and

TABLE 2. Parameters and estimates for the variable juvenile mortality model, North Sea (SE's in parentheses). n is the median number of cohorts used in the analysis.

	Cod	Haddock	Whiting	Plaice	Sole
$l_{t,0,1}$	EGFS	EGFS	EDGS	TRIDENS	TRIDENS
$l_{t,0,2}$	DGFS	SGFS	DGFS	INSHORE	INSHORE
$l_{t,1,1}$	EGFS	EGFS	EGFS	TRIDENS	TRIDENS
$l_{t,1,2}$	DGFS	SGFS	DGFS	INSHORE	INSHORE
n	12	12	12	19	21
λ	0.90	0.89	0.51	0.57	0.28
	(0.11)	(0.06)	(0.23)	(0.11)	(0.17)
ϕ	1.76	0.82	0.43	0.77	1.58
	(0.91)	(0.44)	(0.31)	(0.26)	(0.55)
$\theta_{0,1}$	0.85	0.10	0.33	0.23	0.97
	(0.40)	(0.06)	(0.23)	(0.10)	(0.39)
$\theta_{0,2}$	0.36	0.13	0.67	0.05	0.00
	(0.20)	(0.07)	(0.35)	(0.07)	(0.25)
$\theta_{1,1}$	0.31	0.06	0.05	0.12	0.19
	(0.15)	(0.03)	(0.06)	(0.07)	(0.17)
$\theta_{1,2}$	0.03	0.04	0.43	0.22	0.40
	(0.05)	(0.02)	(0.20)	(0.09)	(0.20)
ψ	–0.14	–0.06	–0.02	0.08	0.73
	(0.11)	(0.03)	(0.07)	(0.06)	(0.27)

TABLE 3. Parameters and estimates for the variable juvenile mortality model, Irish Sea (SE's in parentheses). n is the median number of cohorts used in the analysis.

	Cod	Plaice	Whiting
$l_{t,0,1}$	OCTOBER	OCTOBER	OCTOBER
$l_{t,0,2}$	GADOID	GADOID	GADOID
$l_{t,1,1}$	OCTOBER	OCTOBER	OCTOBER
$l_{t,1,2}$	GADOID	GADOID	GADOID
n	12	14	11
λ	0.61	0.48	1.78
	(0.11)	(0.24)	(5.45)
ϕ	3.70	0.75	0.03
	(1.75)	(0.47)	(0.10)
$\theta_{0,1}$	–0.02	0.53	2.39
	(0.26)	(0.36)	(1.08)
$\theta_{0,2}$	0.88	0.40	0.04
	(0.49)	(0.32)	(0.10)
$\theta_{1,1}$	0.61	0.83	1.15
	(0.37)	(0.44)	(0.52)
$\theta_{1,2}$	0.99	0.58	0.05
	(0.52)	(0.34)	(0.08)
ψ	–0.05	–0.14	–0.05
	(0.26)	(0.21)	(0.32)

TABLE 4. Parameters and estimates for the variable juvenile mortality model, western North Atlantic (SE's in parentheses). n is the median number of cohorts used in the analysis.

	Cod Gulf of Maine		Cod Southern Grand Banks	Cod St. Pierre Bank
$l_{t,0,1}$	FALL	$l_{t,1,1}$	CANADA	CANADA
$l_{t,0,2}$	FALLMA	$l_{t,1,2}$	RUSSIA	FRANCE
$l_{t,2,3}$	SPRING	$l_{t,2,1}$	CANADA	CANADA
$l_{t,2,4}$	SPRINGMA	$l_{t,2,2}$	RUSSIA	FRANCE
n	18	n	17	17
λ	0.21	λ	1.90	0.33
	(0.18)		(4.13)	(0.45)
ϕ	2.26	ϕ	0.39	1.32
	(1.57)		(0.95)	(1.54)
$\theta_{0,1}$	6.33	$\theta_{1,1}$	3.37	3.16
	(2.86)		(1.50)	(1.88)
$\theta_{0,2}$	0.82	$\theta_{1,2}$	2.90	5.22
	(1.27)		(1.35)	(2.48)
$\theta_{2,3}$	1.24	$\theta_{2,1}$	0.01	1.11
	(0.56)		(0.20)	(0.61)
$\theta_{2,4}$	0.34	$\theta_{2,2}$	0.78	1.82
	(0.28)		(0.36)	(0.83)
ψ	0.15	ψ	–0.43	0.03
	(0.26)		(2.90)	(0.48)

IIb. This is a shorter time series than we use for haddock, it begins in 1982, but cod is more widespread than haddock and it appears to give better estimates.

The southern population of cod in the Baltic Sea (Table 5) was surveyed since 1980 by the Federal Republic of Germany (ICES 1991). We treat the cod in Baltic ICES region 22 as a unit population. Although there are surveys in November, January, and March, we use the 0+ November survey and the 1+ survey in January as "simultaneous" measures of 0+ recruit numbers. Similarly, we use the November 1+ and January 2+ surveys as measures of the abundance a year later. That is, we assume that most mortality occurs during the spring, summer, and autumn.

Results

The model was fit to data from 14 populations (Tables 2–5). In only one case, North Sea sole (Table 2), was the interannual variance in juvenile mortality significant at the 0.05 nominal level. Overall it is clear that the estimates of the variability in the density-independent component of juvenile mortality (ψ) are small compared with recruitment variability (ϕ). The estimates of ψ are positive for only 5 of the 14 populations.

We can obtain estimates less than zero because the variance is a component of a larger model. If the interannual variance in juvenile mortality is zero, we would expect about half the estimates to be negative with relatively small sample size.

TABLE 5. Parameters and estimates for the variable juvenile mortality model, Arctic (SE's in parentheses). n is the median number of cohorts used in the analysis.

	Cod Northeast Arctic	Haddock Northeast Arctic		Cod Southern Baltic Sea
$l_{t,1,1}$	RUSSIA	RUSSIA	$l_{t,0,1}$	NOVEMBER
$l_{t,1,2}$	NORWAY	NORWAY	$l_{t,1,2}$	JANUARY
$l_{t,2,1}$	RUSSIA	RUSSIA	$l_{t,1,1}$	NOVEMBER
$l_{t,2,2}$	NORWAY	NORWAY	$l_{t,2,2}$	JANUARY
n	10	23	n	10
λ	1.09	0.91	λ	1.76
	(0.32)	(0.15)		(0.78)
ϕ	2.76	4.38	ϕ	3.14
	(1.87)	(1.78)		(2.51)
$\theta_{1,1}$	1.57	0.13	$\theta_{0,1}$	4.24
	(0.91)	(0.34)		(2.07)
$\theta_{1,2}$	3.08	1.17	$\theta_{1,2}$	4.08
	(1.55)	(0.57)		(2.01)
$\theta_{2,1}$	1.15	0.37	$\theta_{1,1}$	1.96
	(0.64)	(0.27)		(1.07)
$\theta_{2,2}$	1.55	0.20	$\theta_{2,2}$	1.44
	(0.81)	(0.24)		(0.89)
ψ	−1.16	0.92	ψ	−3.60
	(0.77)	(0.51)		(3.35)

The density-dependent component of survival appears to be important for most populations. For cod, λ is less than 1 for most populations. An exception is southern Grand Banks cod and southern Baltic Sea cod. In both of these cases the observational error variance is quite high, and none of the estimates are reliable.

Power and Bias Analysis

Our interest here is to quantify the power of the test that interannual variance in juvenile mortality, ψ, is 0, i.e., H_0: $\psi = 0$ versus H_a $\psi > 0$ (Peterman 1990). Specifically, for a given value of ψ (we choose $\psi = 0, 0.1, 0.25, 0.5$, and 0.9), we are interested in the small sample probability that the test rejects H_0 at different levels of the estimation error variance (θ) in favor of H_a (indicating significant interannual variance in juvenile mortality).

The power analysis is performed by generating 1000 realizations of the model with $\phi = 1$, $\lambda = 0.8$, θ as defined in Fig. 3, and the number of years of data used, n, to be either 10 or 20. Thus, we generate 1000 data sets, each with n observations of four variables, for each of the 15 models considered. For each data set and for each particular model, we estimate the parameters and test H_0. For each model, we then count the number of times H_0 is rejected in the 1000 realizations; the simulated power of the test is this number divided by 1000.

There are two ways we considered testing the two-sided alternative of the above hypothesis. In the likelihood ratio test the model is fit twice: first with ψ estimated and second with ψ constrained to be zero. If the reduction in the estimated log-likelihood in the second case is greater than $X^2_{[1]}/2$, the parameter is considered significant (Cox and Hinkley 1974). The second approach is to test the significance of ψ by using the estimated standard error and the assumption that the estimate of ψ follows a normal distribution — an asymptotic result. We found the power of both tests to be virtually identical. Because the likelihood ratio test is difficult to apply for the one-sided alternative, we only use and present the results from the second test.

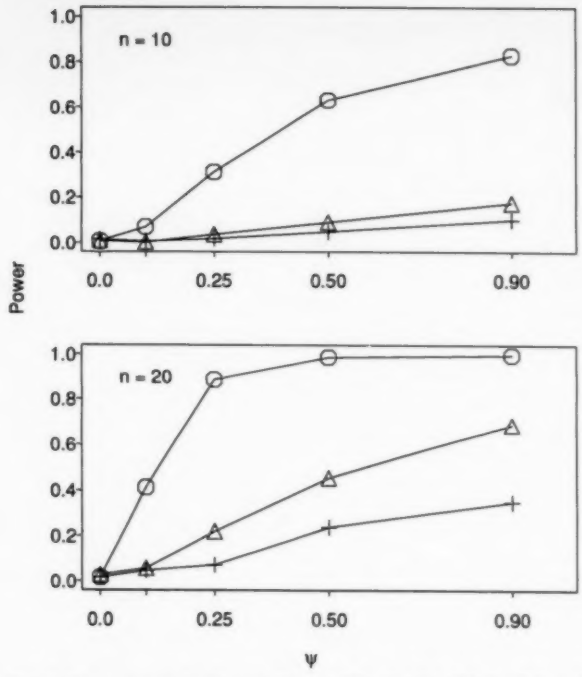


FIG. 3. Power curves for the test H_0: $\psi = 0$ for $\theta = 0.1$ (○), $\theta = 0.5$, and (△), and $\theta = 1.0$ (+) where θ is the estimation error variance and ψ is the interannual variance of the density-independent juvenile mortality. See text for details.

TABLE 6. Simulated %bias of the maximum likelihood estimate of ψ. Eighteen sets of simulations were conducted with data generated in a $2 \times 3 \times 3$ factorial experiment with two sample sizes (n), three values of the estimation error variance (θ), and three values of the variance in juvenile mortality unrelated to abundance (ψ). The variance in recruitment (ϕ) is always equal to 1 and the density-dependent survival rate (λ) is always equal to 0.8.

		ψ		
n	θ	0.25	0.50	0.90
20	0.01	−12.1	−11.6	−8.4
	0.50	−20.6	−12.4	−18.9
	1.00	−60.4	−31.0	−31.5
10	0.01	−30.9	−23.3	−30.5
	0.50	−101.8	−41.3	−41.9
	1.00	−163.3	−79.5	−79.7

The hypothesis was tested with a type I error (α) of 0.05. When $\psi = 0$ the simulated α is less than 0.05 (see Fig. 3), indicating that the test is conservative at the sample sizes studied. Otherwise, if the estimation error (θ) decreases the power of the test increases. Also, the test is not very powerful at rejecting H_0 when ψ is small and when θ is large in comparison with ϕ.

In conjunction with the power analysis, the biases in parameter estimates were also investigated. The results for density-

dependent mortality parameters and the estimation error variances are similar to those obtained in Myers and Cadigan (1993) and are thus not discussed here. The bias in the maximum likelihood estimate of ψ is generally negative (Table 6). The %bias decreases with sample size and the true ψ and increases with the error variances. This negative bias results in a reduced statistical power in detecting significant ψ. If the estimation error variance is less than half the ϕ for sample sizes of around 10 the results cannot be interpreted because of the large bias in the estimated ϕ.

Considerable effort has been invested into producing a bias reduced estimator for the type of model considered here. A resampling procedure (the jackknife) and an analytic procedure have been used and simulations conducted to assess their usefulness. The simulation results are too extensive to report but they do not suggest that either bias-reduced estimator works well. Hence, we have no better estimator of ψ than the maximum likelihood estimator used.

Violations of the Model Assumptions

A violation of the model assumptions could bias our results. An environmental factor that simultaneously influenced the efficiency of the survey gear in capturing fish in both surveys would result in an overestimate of variability of juvenile mortality, ψ. Given the general absence of evidence for positive ψ, this factor is probably not of general importance, but it cannot be eliminated for North Sea sole.

It is possible that other violations of the model assumptions could have biased our results. In Myers and Cadigan (1993), we tested the bias in a similar model that would result if recruitment and the estimation errors followed other distributions than assumed. In no case were the resulting biases important. We therefore believe that reasonable violations of these assumptions are not important for our conclusions.

Discussion and Conclusions

We have provided a systematic test of one portion of Hjort's hypothesis: we estimated the proportion of recruitment variability caused by variable juvenile survival.

Our results (Tables 2–5) should be interpreted in light of the simulation studies. The average estimation error variance is more than half of ϕ for the following populations: North Sea whiting, Irish Sea plaice, Irish Sea whiting, Gulf of Maine cod, southern Grand Banks cod, St. Pierre Banks cod, Northeast Arctic cod, and southern Baltic Sea cod. The bias in estimating the interannual variance in juvenile mortality appears to be too large for the results to be interpreted because of the relatively large estimation error variance.

The results for the other six populations appear to be reliable because the estimation error variance is relatively small. The estimates of ψ are positive for only two of these populations. Overall the results indicate that we would have detected an effect in more populations than one if the density-independent component of juvenile mortality was highly variable.

The results for North Sea sole appear to be reliable. There is previous evidence of interannual variability in postlarval survival in this flatfish, particularly related to extreme cold or freezing (Woodhead 1964; de Veen 1969). The strong correlations between the surveys at age 0 and at age 1 (Fig. 1) appear to be reliable in the sense that they would remain strong even if the most influential points were removed from the analysis. For this population the interannual variability in juvenile survival appears to be the most important source of variability in abundance. The log variance of numbers at age 1 will be $\lambda^2\phi + \psi$. By substituting the estimates from Table 2, we can estimate for North Sea sole that approximately 85% of the interannual variance in log abundance at age 1 is caused by variable density-independent mortality in the juvenile stage.

The only other population with evidence of density-independent variability in juvenile survival is Northeast Arctic haddock. Although the estimate of the ψ was close to being statistically significant, it does not appear to account for a large portion of the interannual variance at age 1.

Our results indicate that it is very difficult to detect density-independent variability in juvenile survival. Research programs that attempt to investigate this portion of the life history would need to have low estimation error variance and must be long term, i.e., 20 yr. The value of short-term studies for estimating the importance of the juvenile stage in recruitment studies must be questioned.

Fisheries scientists often have access to a plethora of population dynamics data. Too often these data are not examined to ask fundamental ecological questions. In this paper and in Myers and Cadigan (1993), we have shown how modern statistical methods can be used to address these questions.

Our results suggest that the variability of relative year class strength is usually determined in the larval stage for marine demersal species (see also Bradford 1992) but that this variability is attenuated by density-dependent juvenile mortality (Myers and Cadigan 1993). For North Sea sole, variable mortality of juveniles may also contribute to recruitment variability. This study represents one of a few attempts to directly test Hjort's hypothesis that the variability in year class strength is determined in the larval stage. Although our results support Hjort's hypothesis in general, our results indicate two important modifications: (1) variable mortality of juveniles may be important in some flatfish species and (2) variability in recruitment created in the larval stage is attenuated by density-dependent mortality in the juvenile stage.

Acknowledgements

We thank Jim Baird, Keith Brander, Ray Conser, Tore Jakobsen, Wendy Gabriel, Andy Rosenberg, and Mikel Hilden for assistance in understanding the data sets. We thank M. Bradford, Rick Deriso, and C. Walters for helpful comments on the method.

References

Bishop, C.A., J.W. Baird, and E.F. Murphy. 1991. An assessment of the cod stock in NAFO Subdivision 3Ps. CAFSAC Res. Doc. 91/36.

Bollen, K.A. 1989. Structural equations with latent variables. Wiley, New York, N.Y. 541 p.

Bradford, M.J. 1992. Precision of recruitment predictions from early life stages of marine fishes. Fis. Bull. 90: 439–453.

Cox, D.R., and D.V. Hinkley. 1974. Theoretical statistics. Chapman and Hall, London. 210 p.

de Veen, J.F. 1969. Vlekziekte en dode tng n de Duitse Bocht in het voorjaar van 1969. Visserij 22: 482–487.

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 20: 1–228.

Hjort, J. 1926. Fluctuations in the year classes of important food fishes. J. Cons. 1: 1–38.

ICES. 1991. Report of the working group on assessment of demersal stocks in the Baltic. ICES Doc. C.M. 1991/Assess:16.

May, R.M. [ed.] 1984. Exploitation of marine communities. Life Sci. Res. Rep. 32. Springer-Verlag, Berlin.

MOGUEDET, P. 1991. Contribution to the assessment of the cod stock in Subdivision 3Ps. NAFO SCR Doc. 91/5. Ser. No. N1903.

MYERS, R.A., W. BLANCHARD, AND K.R. THOMPSON. 1990. Summary of North Atlantic fish recruitment 1942–1987. Can. Tech. Rep. Fish. Aquat. Sci. 1743: 108 p.

MYERS, R.A., AND N.G. CADIGAN. 1993. Density-dependent juvenile mortality in marine demersal fish. Can. J. Fish. Aquat. Sci. 50: 1576–1590.

PETERMAN, R.M. 1990. Statistical power analysis can improve fisheries research and management. Can. J. Fish. Aquat. Sci. 46: 2–15.

PETERMAN, R.M., M.J. BRADFORD, N.C.H. LO, AND R.D. METHOT. 1988. Contribution of early life stages to interannual variability in recruitment of northern anchovy (*Engraulis mordax*). Can. J. Fish. Aquat. Sci. 45: 8–16.

SMITH, P.E. 1985. Year-class strength and survival of O-group clupeoids. Can. J. Fish. Aquat. Sci. 42(Suppl. 1): 69–82.

WOODHEAD, P.M.J. 1964. The death of north Sea fish during the winter 1962/63, particularly with reference to the sole, *Solea vulgaris*. Helgol. Wiss. Meeresunters. 10: 283–300.

Salinity and Recruitment of Atlantic Cod (*Gadus morhua*) in the Newfoundland Region

Ransom A. Myers

Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada

Kenneth F. Drinkwater

Department of Fisheries and Oceans, Physical and Chemical Sciences Branch, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada

Nicholas J. Barrowman

Seaconsult Ltd., P.O. Box 2035, Station C, 200 White Hills Road, St. John's, NF A1C 5R6, Canada

and James W. Baird

Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada

Myers, R.A., K.F. Drinkwater, N.J. Barrowman, and J.W. Baird. 1993. Salinity and recruitment of Atlantic cod (*Gadus morhua*) in the Newfoundland region. Can. J. Fish. Aquat. Sci. 50: 1599–1609.

Recruitment predictions for Atlantic cod (*Gadus morhua*) in the North Atlantic, based upon a previously published regression with salinity, are found to be well correlated with recent recruitment estimates from both virtual population analysis and those derived from research surveys. The addition of spawning stock biomass in the regression significantly increased the percentage of the variance accounted for in the recruitment time series. A similar relationship between recruitment and salinity was found for two nearby stocks (southern Grand Banks and St. Pierre Bank). Oceanographic and food chain mechanisms that might be responsible for a link between salinity and recruitment are discussed.

Les prédictions du recrutement chez la morue franche (*Gadus morhua*) du nord de l'Atlantique, fondées sur une régression antérieurement publiée selon la salinité, se révèlent bien corrélées avec les estimations récentes du recrutement découlant de l'analyse des populations virtuelles et des relevés de recherche. L'adjonction de la biomasse des stocks de géniteurs dans la régression a notablement augmenté le pourcentage de la variance expliquée par les séries chronologiques du recrutement. Une relation semblable entre le recrutement et la salinité a été constatée pour deux stocks voisins (du sud des Grands Bancs et du banc de Saint-Pierre). Nous discutons des mécanismes océanographiques et trophiques qui pourraient expliquer le lien entre la salinité et le recrutement.

Received January 20, 1993
Accepted April 28, 1993
(JB760)

Reçu le 20 janvier 1993
Accepté le 28 avril 1993

In 1992, the low numbers of commercial-size Atlantic cod (*Gadus morhua*) occupying the waters off southern Labrador, northern Newfoundland, and the northern Grand Banks (Northwest Atlantic Fisheries Organization (NAFO) subareas 2J3KL; Fig. 1) resulted in the Canadian government imposing a 2-yr moratorium on its cod fishery. Although the exact causes of the decline in cod abundance are uncertain, it is clear that early predictions of recruitment would facilitate better long-term management of the stock. Present methods rely upon research surveys that forecast recruitment based upon numbers of cod of age 3 or 4, i.e., only 2–3 yr before the fish are fully recruited into the fishery (age 6). The traditional survey methods are unable to provide earlier predictions because of the different behavior and spatial distributions of the young fish compared with the older adults which are the primary target of the surveys. However, earlier predictions may be possible if recruitment can be shown to be related to other, more easily measured variables.

One such relationship has been published. Sutcliffe et al. (1983) found a strong correlation between 2J3KL cod recruitment and the 0–50 m depth-averaged summer (July–September) salinity at Station 27, a hydrographic site off St. John's, Newfoundland (Fig. 1). They cast the relationship in terms of the multiple linear regression equation

$$(1)\quad \text{Recruitment}_y = -26360 + 162s_y + 278s_{y+1} + 406s_{y+2}$$

where Recruitment_y is the number of cod produced in year y (estimated as 4-yr-olds) and s_y is salinity in year y. Recruitment is in numbers ($\times 10^6$) although in the original paper, it was mistakenly listed as ($\times 10^5$). In the original analysis, salinity was assumed to affect survival during the first 3 yr of life. The recruitment series Sutcliffe et al. (1983) used was 1958–76 from a virtual population analysis (VPA) by Wells and Bishop (1980).

Sutcliffe et al. (1983) suggested that the interannual variability of cod recruitment arose from the variation in its food supply, which was linked to fluctuations in mixing within Hudson Strait and the subsequent surface nutrient flux onto the Labrador Shelf. They suggested that high runoff from Hudson Strait "caps" the tidal mixing outside the strait which inhibits nutrient pumping into the surface layer and reduces productivity. This hypothesis thus provided a rationale for the positive relationship between salinity and recruitment. In support of this hypothesis, they found

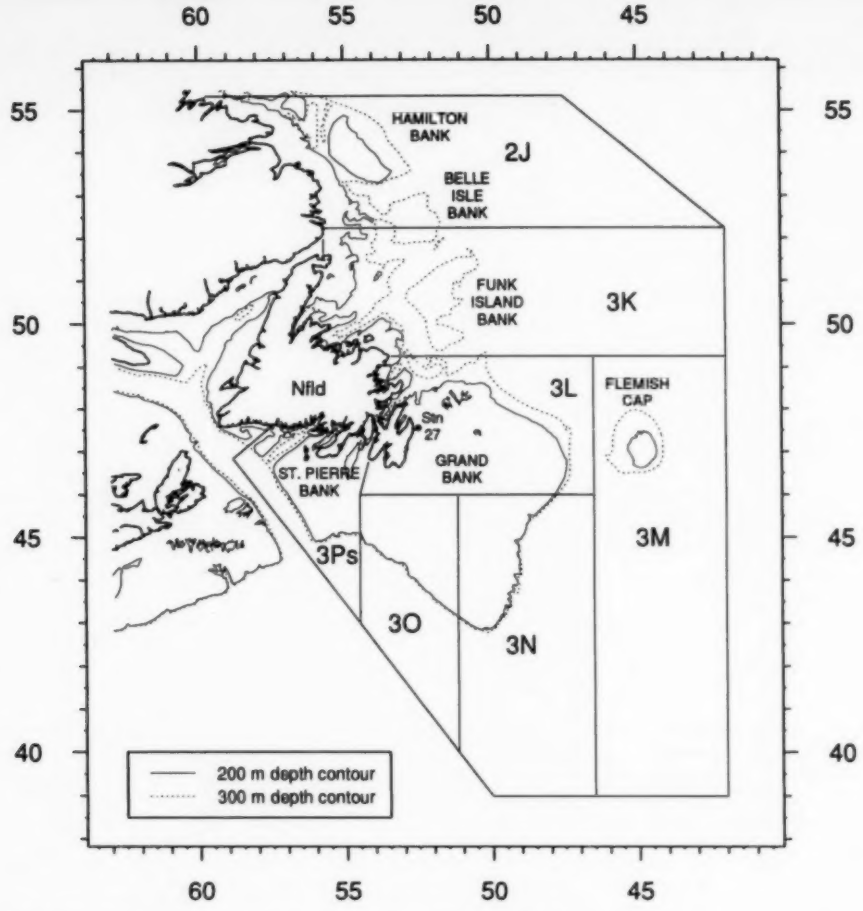


FIG. 1. Newfoundland region showing the locations of NAFO divisions and Station 27.

an inverse relationship between the seasonal cycles in runoff into Hudson Bay and the depth-averaged salinity in the upper 50 m at Station 27 when the salinity lagged the runoff by 4 mo, a time scale consistent with what was known about oceanic advection in the region at that time. They speculated on the possibility of a relationship at interannual time scales but lacked the necessary time series of freshwater discharge from the Hudson Bay region to test this hypothesis directly. Recent studies (Myers et al. 1990) have indicated that the seasonal and interannual variability in salinity at Station 27 is more closely related to sea-ice extent over the Labrador and northern Newfoundland shelves and that Hudson Bay runoff is only weakly correlated with salinity on the Newfoundland Shelf.

In this paper, we reexamine the relationship between salinity and recruitment of 2J3KL cod. We first compare the predictions, based upon the regression relationship from Sutcliffe et al. (1983), with the recent VPA recruitment estimates. Since VPA methods tend to smooth the recruitment time series, we further test the relationship using estimates of cod recruitment derived from research surveys. We also investigate the effects of spawning stock biomass on recruitment of 2J3KL cod. The analysis is then extended to three nearby stocks (southern Grand Banks: NAFO Division 3NO; St. Pierre Bank: NAFO Division 3Ps; Flemish Cap: NAFO Division 3M) because it was felt that similar mechanisms may be operating in these regions. Finally, we consider oceanographic and food chain mechanisms that may be responsible for the link between salinity and recruitment.

Data and Statistical Methods

Salinity

The depth-averaged (0–50 m) summertime (July, August, and September) salinities at Station 27 (Fig. 1) were determined each year for the period 1946–92 using linear interpolation between depths. The interannual variability in the summertime salinity is dominated by a maximum in the mid-1960's, a rapid decline to a minimum in the early 1970's, followed by variability superimposed upon a steady rise through to 1990 (Fig. 2).

Recruitment Indices

The 1958–87 recruitment estimates for 2J3KL cod (Fig. 2), based upon the numbers of age 4 cod from a VPA calibrated with the Canadian fall research survey estimates of abundance, were

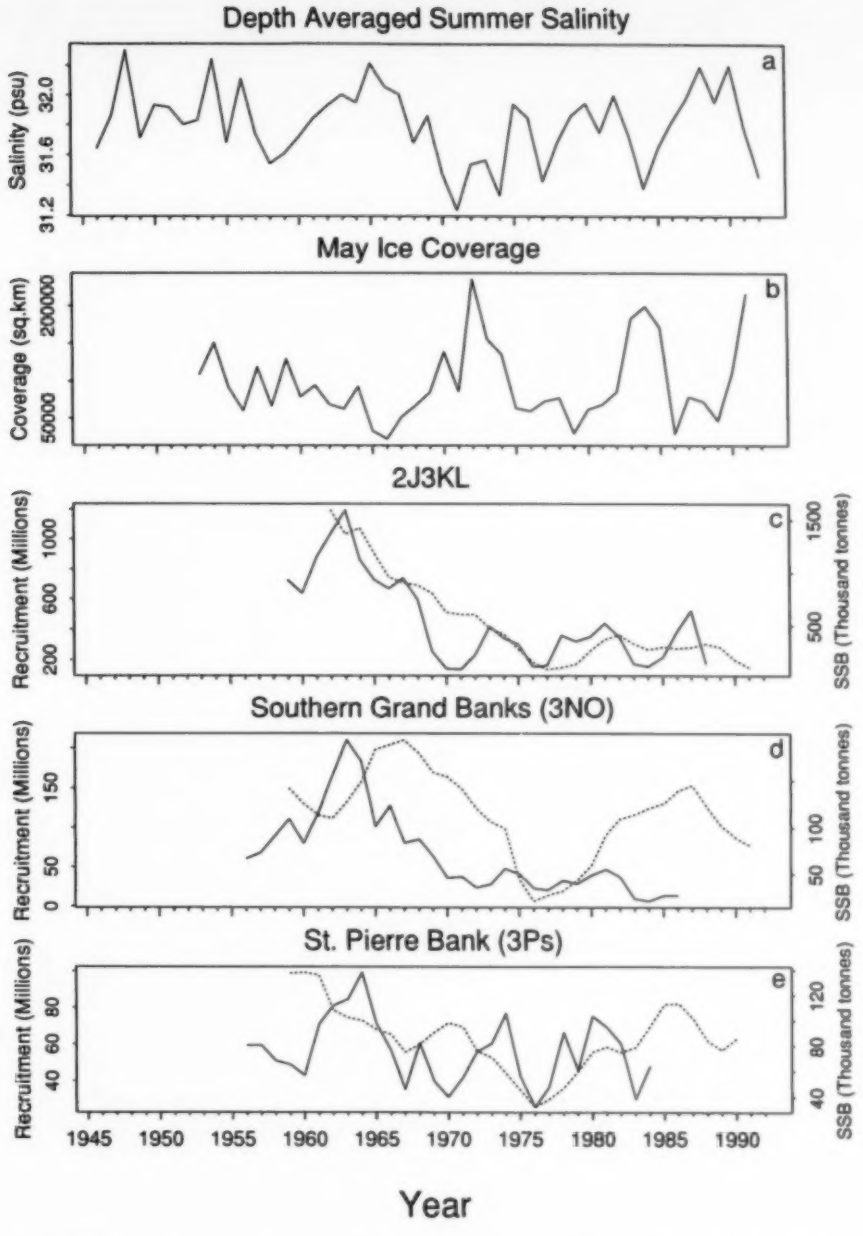


FIG. 2. (a) Yearly depth-averaged summer (July–September) salinity at Station 27 from 0 to 50 m. (b) Ice cover south of 55° along the Labrador–Newfoundland coast in May. (c–e) Recruitment (—) and spawning stock biomass (• • •) from VPA for 2J3KL cod, southern Grand Banks cod, and St. Pierre Bank cod.

taken from Baird et al. (1992a). Similar VPA estimates were also examined for the southern Grand Banks (3NO) calibrated with the Canadian and Russian spring research surveys (1959–86; Baird et al. 1992b), St. Pierre Bank (3Ps) (1959–87; Bishop et al. 1991) calibrated with the Canadian and French spring research surveys, and Flemish Cap (3M) (1956–84; Wells et al. 1984) (Fig. 2) calibrated with the Canadian spring research survey. Note that the recruitment estimate for a given year is determined from the commercial catches 4 yr later, e.g., the 1991 catch data provided the recruitment estimate for 1987. Except for direct comparisons with Sutcliffe et al. (1983), we used the recruitment estimated at age 3, which is generally available. Estimates of spawning stock biomass (SSB) were also obtained from VPA (Fig. 2). Age at maturity was not available for all years; therefore, we used average ages of maturity. This unfortunately will introduce some errors into the analysis, but they should be relatively small. For 2J3KL cod, we used the biomass of fish of age 7 and older at the beginning of the year. For the southern

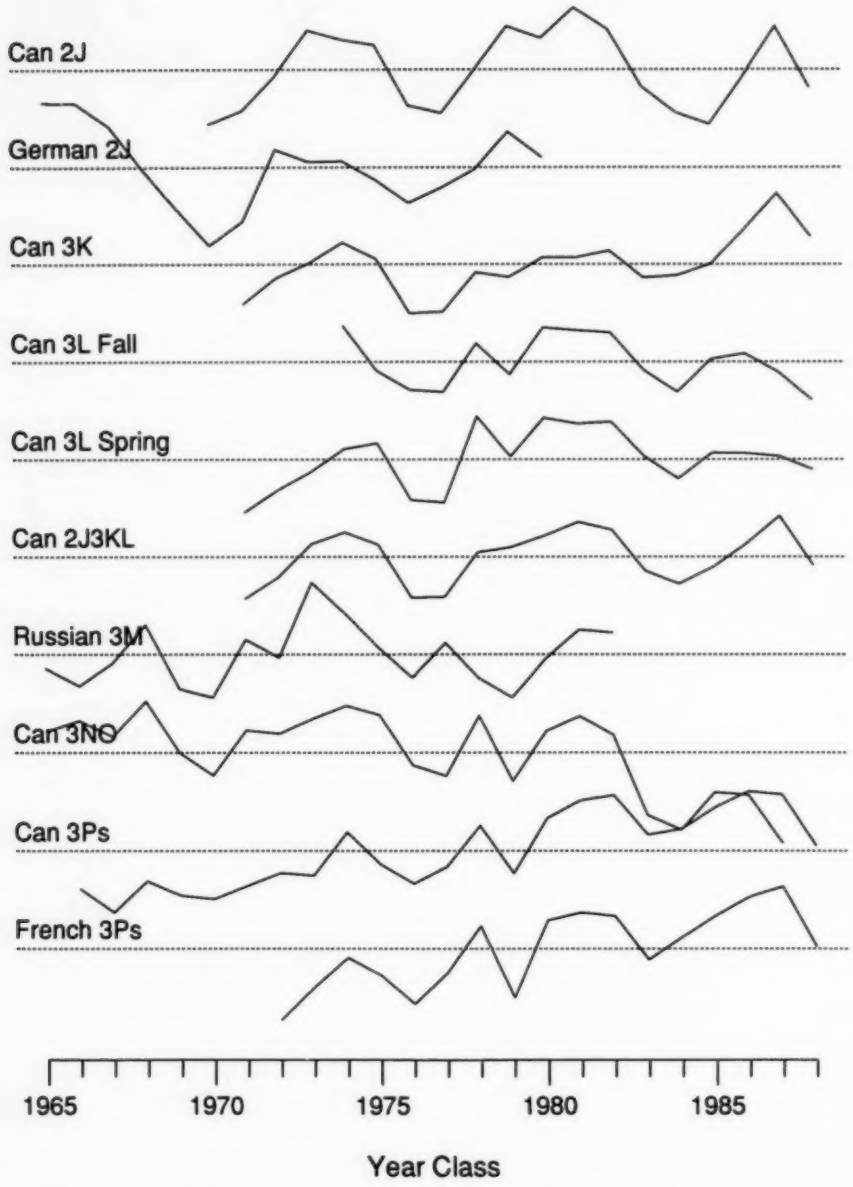


FIG. 3. Recruitment time series for various research surveys around Newfoundland. Estimates of numbers at age in a year class are log transformed (base 10) with the mean removed. The mean of each series is separated by 1 unit, i.e., a factor of 10, from the one below. Thus, the distance between the dotted lines gives the vertical scale.

Grand Banks (3NO) and St. Pierre Bank (3Ps), we used the average maturity at age given in Baird et al. (1986) and Bishop (1984). The beginning of year biomass was used in all cases.

One difficulty in using VPA results is that year classes of recruitment tend to be smeared together. This can make recruitment studies relying upon year-to-year variability difficult to sort out. Because of this problem, we supplemented the VPA recruitment estimates with indices derived solely from research

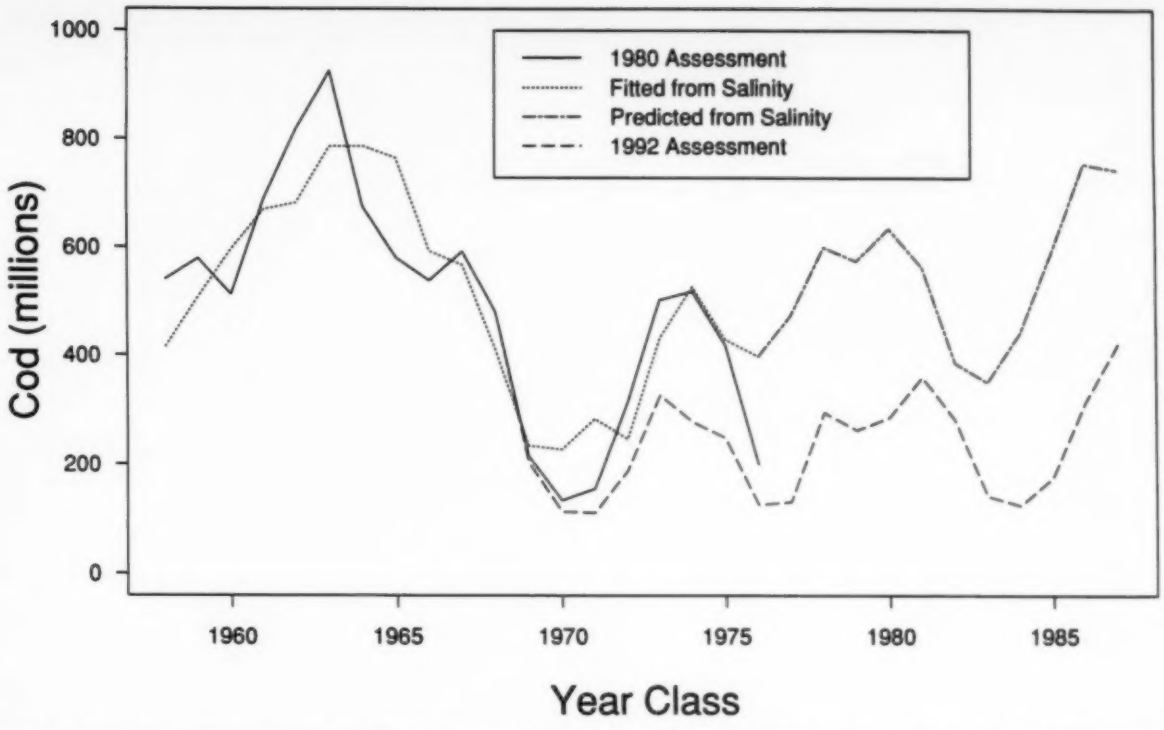


FIG. 4. The 1980 assessment estimates for 2J3KL cod recruitment at age 4 (—) and the fitted values in the original regression (Eq. 1) proposed by Sutcliffe et al. (1983) with summer salinity at Station 27 (• • •). Also shown is the 1992 assessment of cod (– – –) and the predicted recruitment from 1975 to 1987 using the salinity regression results (– - –).

surveys. To extract the survey effects at age, we have used a modification of a multiplicative catch-at-age model developed by Shepherd and Nicholson (1991). A description of the method follows.

Let the numbers of fish of age a in year y be $N_{y,a}$ and consider only ages at which commercial fishing mortality is small. Let P_a, the survival to age a, be constant. Myers and Cadigan (1991, 1993) showed that for cod, haddock (*Melanogrammus aeglefinus*), plaice (*Pleuronectes platessa*), whiting (*Merlangius merlangus*), and American plaice (*Hippoglossoides platessoides*) the interannual variation in juvenile natural mortality unrelated to density was small. Let the number of recruits in year y be $N_{y,0}$. The numbers at age will then be

$$(2) \quad N_{y,a} = P_a N_{y-a,0}.$$

Let the number of fish of age a in year y estimated from survey i be $R_{y,a,i}$. Assume that the estimate of abundance is proportional to the true abundance, i.e., the catchability of survey i at age a is $Q_{a,i}$. The error, $e^{\varepsilon_{y,a,i}}$, appears in the model multiplicatively. Thus

$$(3) \quad R_{y,a,i} = Q_{a,i} N_{y,a} e^{\varepsilon_{y,a,i}}$$

$$(4) \quad = Q_{a,i} P_a N_{y-a,0} e^{\varepsilon_{y,a,i}}$$

where $\varepsilon_{y,a,i}$ is assumed to be normal with constant variance. Using a log transformation, and letting lowercase letters represent the log transform of parameters and variables, the last equation becomes

$$(5) \quad r_{y,a,i} = q_{a,i} + p_a + n_{y-a,0} + \varepsilon_{y,a,i}.$$

Note that $q_{a,i}$ and p_a are confounded in the above equation and therefore, we only attempt to estimate the sum of these two parameters. If the same survey vessel was used throughout the survey period, we estimated a year class effect for each year class, $n_{y-a,0}$, and age effect for each age used in the analysis, which is the sum of $q_{a,i}$ and p_a. In the Canadian spring surveys for 3L, southern Grand Banks (3NO), and St. Pierre Bank (3Ps), there was a change in survey vessel in year 1983. We estimated separate age effects for each survey vessel because they did not fish with the same efficiency.

The above equation is a simple analysis of variance model with year class and age effects. If the error variance is approximately constant, then the year class effects can be estimated using any standard analysis of variance programs. The estimates of the year class effects, $n_{y,0}$, (on a log scale), are used as relative recruitment indices (Shepherd and Nicholson 1991). Ages 2–6 were used from the fall surveys and ages 2–7 from the spring surveys. The exception was the USSR (3M) survey for which only ages 1–3 were available.

Data from a variety of research vessel surveys covering NAFO subareas 2J, 3K, and 3L were available (Fig. 3). For 2J, we analyzed the Federal Republic of Germany (1972–83) and the Canadian (1977–91) autumn surveys. There is excellent agreement between the two during the period of overlap. For 3K, Canadian autumn surveys were available from 1978 to 1992. These match closely the 2J indices. For 3L, there were Canadian surveys during both the spring (1977–82, 1985–92) and autumn

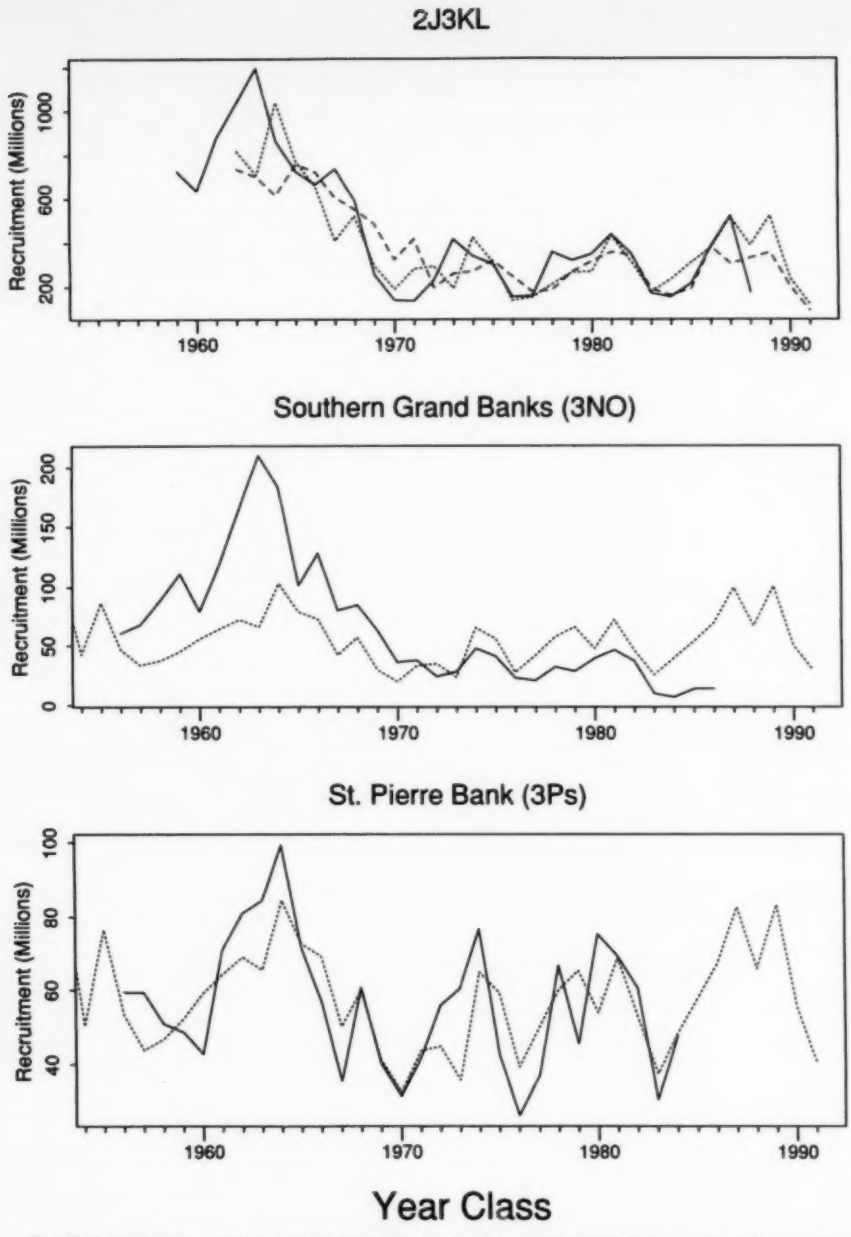


FIG. 5. Recruitment at age 3 estimated from VPA (—) and the results from the regression of log recruitment on log SSB and salinity at age 1 (• • •) for 2J3KL cod and salinity at age 1 for St. Pierre Bank (3Ps) and southern Grand Banks (3NO) cod. For 2J3KL cod, we also show the regression results for log SSB and May sea-ice cover south of 55° (- - -).

(1981–91). The 3L recruitment indices derived from the different seasonal surveys are similar; however, there were consistent differences with the 2J and 3K surveys. For example, the 1978 year-class was strong in 3L but not in 2J.

We also obtained research vessel survey data for other NAFO subareas (see Fig. 1 for locations). The southern Grand Banks (3NO) was surveyed each spring by Canada from 1971 to 1992. Both Canada (1972–92) and France (1972–89) have conducted spring surveys in the vicinity of St. Pierre Bank (3Ps). The survey-derived recruitment indices for both the southern Grand Banks (3NO) and St. Pierre Bank (3Ps) show similarities to those for 3L and 2J (Fig. 3). The Flemish Cap area (3M) was surveyed by the USSR from 1962 to 1982 but it showed different recruitment trends from those in the other regions. The short research survey series of the Flemish Cap by Canada (1978–82) and by the EEC (1989–92) were not used in our analysis.

TABLE 1. Results of a stepwise regression of log-transformed VPA recruitment estimates on log-transformed spawning stock biomass (SSB), salinity in the year of hatch (sal), salinity in the following year (sal1), and salinity 2 yr later (sal2) for divisions 2J3KL, 3NO, 3Ps, and 3M.

Division	Variables	Year classes	r^2	Nominal p-value
2J3KL	sal1 SSB	1962–88	0.680	0.0001
3NO	sal1	1959–86	0.258	0.0071
3Ps	sal1	1959–87	0.537	0.0001
3M	sal2	1956–84	0.034	0.3566

TABLE 2. Results of a stepwise regression of year class estimates from research surveys on salinity in the year of hatch (sal), salinity in the following year (sal1), and salinity 2 yr later (sal2). Note that only sal1 was selected in the stepwise regression with the exception of the 3M surveys.

Division	Variable selected	Year classes	r^2	Nominal p-value
Canadian 2J	sal1	1970–88	0.240	0.0332
German 2J	sal1	1965–80	0.534	0.0013
Canadian 3K	sal1	1971–88	0.493	0.0012
Canadian 3L spring	sal1	1971–88	0.240	0.0391
Canadian 3L fall	sal1	1974–88	0.072	0.3354
Canadian 2J3KL	sal1	1971–88	0.393	0.0054
Russian 3M	sal	1961–82	0.157	0.0680
Canadian 3NO	sal1	1967–85	0.131	0.1285
Canadian 3Ps	sal1	1966–88	0.266	0.0118
French 3Ps	sal1	1972–88	0.360	0.0108

Results

Comparison of Predicted and VPA-Estimated Recruitment for 2J3KL Cod

The 2J3KL cod recruitment estimates based upon the salinity regression in Sutcliffe et al. (1983) are shown in Fig. 4 together with the VPA population estimates from the 1980 and 1992 assessments. The 1980 VPA estimates were those used by Sutcliffe et al. (1983) to establish the regression. The salinity-derived estimates are divided into two periods: the regression years (1958–76) and the predicted years (1977–87).

There is a close relationship between the predicted recruitment and the population estimates. The correlation (0.70) was nominally significant ($p = 0.05$); the significance levels have been corrected for autocorrelation according to Bayley and Hammersley (1946) and Garrett and Toulany (1981). Note that the population values for 1970-76 used by Sutcliffe et al. (1983) are larger than the 1992 estimates. This is due to underestimates of the fishing mortality in the 1980 assessment. The overestimates of the populations in the 1970's are believed to have led, in part, to the absolute difference between the predicted and 1992 VPA-estimated recruitment. Another factor that may explain this difference is the decline in spawning stock biomass that occurred after 1970.

Effects of Spawning Stock Biomass on 2J3KL Cod Recruitment

To examine the possible influence of SSB, we performed a stepwise regression with recruitment being a function of SSB and salinity (Table 1). We log transformed the recruitment and SSB estimates to stabilize variance and to linearize the stock-recruitment relationship. The recruitment time series covered the period 1962–88 (birth dates). Correlations between SSB and salinity, either in the year of hatch or at age + 1 or age + 2, were low ($r < 0.3$) and nonsignificant.

The SSB for 2J3KL cod was high in the 1960's, declined sharply in the early 1970's, and has fluctuated about relatively low levels during the last two decades (Fig. 2). Regressing the log recruitment against SSB resulted in an r^2 of 0.38. The relationship was primarily due to extremes (Fig. 2), i.e., when SSB was high (low), recruitment was high (low). The best one-variable model in terms of recruitment variance accounted for was, however, with salinity at age + 1 ($r^2 = 0.42$). Salinity in the year of hatch ($r^2 = 0.32$) and at age + 2 ($r^2 = 0.13$) accounted for less variance. Only the correlation with salinity at age + 1 was significant at the $p < 0.1$ level after correcting for autocorrelation in the time series.

Combining the SSB and salinity at age + 1 accounted for 68% of the recruitment variability. Using salinity only (at age + 1 and in year of hatch) accounted for 50% of the recruitment variability. The relatively small increase in variance accounted for using the 2 years of salinity instead of salinity at age + 1 alone is because of the strong autocorrelation in the salinity time series (e.g., $r = 0.5$ at a lag of 1 yr). The three-variable models using SSB, salinity at age + 1, and salinity in year of hatch accounted for 71% of the recruitment variability, but this is not statistically better than the two-variable model with SSB and salinity at age + 1 because of the loss of degrees of freedom due to the addition of an extra variable.

Based upon our analysis using the VPA estimates, the regression that provides the most reliable prediction of recruitment is

$$\log(\text{Recruitment}_y) = -40.42889 + 0.4281 \log(\text{SSB}_y) + 1.3741 s_{y+1}$$

where recruitment in year y is in millions of cod at age 3, SSB in year y is in thousands of metric tons (tonnes), and s_{y+1} is salinity in year $y + 1$. Note that natural logarithms are used. We have used this relationship and recent salinity and SSB estimates to predict cod recruitment during 1989–91. These are estimated to be weak, with the 1991 recruitment estimated to be the lowest on record (Fig. 5).

We also investigated the relationship between salinity at age + 1 and recruitment for the Flemish Cap area (3M), the southern Grand Banks (3NO), and the St. Pierre Bank (3Ps). For the southern Grand Banks (3NO) and St. Pierre Bank (3Ps) the correlations were positive but significant only for the St. Pierre Bank (3Ps). Recruitment was regressed against salinity at age + 1 for the southern Grand Banks (3NO) and St. Pierre Bank (3Ps) stocks and the results are plotted in Fig. 5.

Research Survey Estimates of Recruitment

We repeated the stepwise regression for the research survey recruitment indices but did not include the effects of SSB because the indices were generally available only after the large decline in biomass in the early 1970's (Table 2). Salinity at age + 1 was the most important variable in each of the nine surveys considered for the 2J3KL, southern Grand Banks (3NO), and St. Pierre Bank (3Ps) stocks. Salinity at other ages did not enter at a nominal significance level of 0.15. For the Flemish Cap area

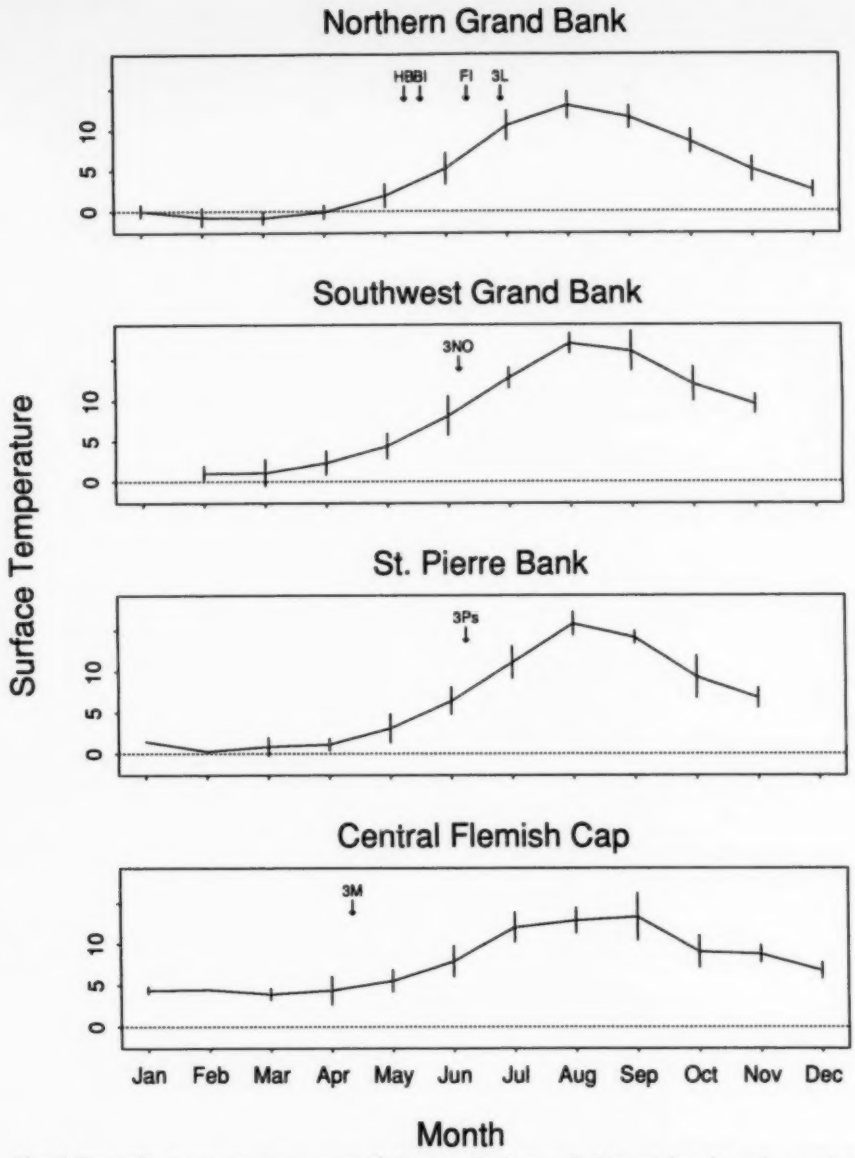


FIG. 6. Sea surface temperature versus month for several subareas. Peak larval days for various regions are also indicated with arrows and the labels HB (Hamilton Bank), BI (Belle Isle Bank), and FI (Funk Island Bank) and the NAFO region labels.

(3M) the relationship was negative, low, and not significant. Table 1 clearly shows that the results for 2J3KL cod using the VPA are not caused by artifacts or errors in the assumptions of the VPA because they are replicated by five independent surveys as well as the combined 2J3KL fall surveys. It is to be expected that the values of r^2 using the research survey estimates are lower than those using the VPA estimates because the former have greater sampling variability.

Although the results for the southern Grand Banks (3NO) are positive, the r^2 is so low that the analysis brings into question the results for the stepwise regression using the VPA. The values of r^2 for St. Pierre Bank (3Ps) are both higher and lend support to the results in Table 1.

Possible Mechanisms

Our results have confirmed the relationship between 2J3KL cod recruitment and salinity found by Sutcliffe et al. (1983), but the question of the underlying mechanism remains. We examine two possibilities.

Food Chain Hypotheses

Sutcliffe et al. (1983) suggested that the link between salinity and recruitment was through the food chain, with high salinity corresponding to high nutrients, high primary and secondary production, and hence more food for cod. However, recent

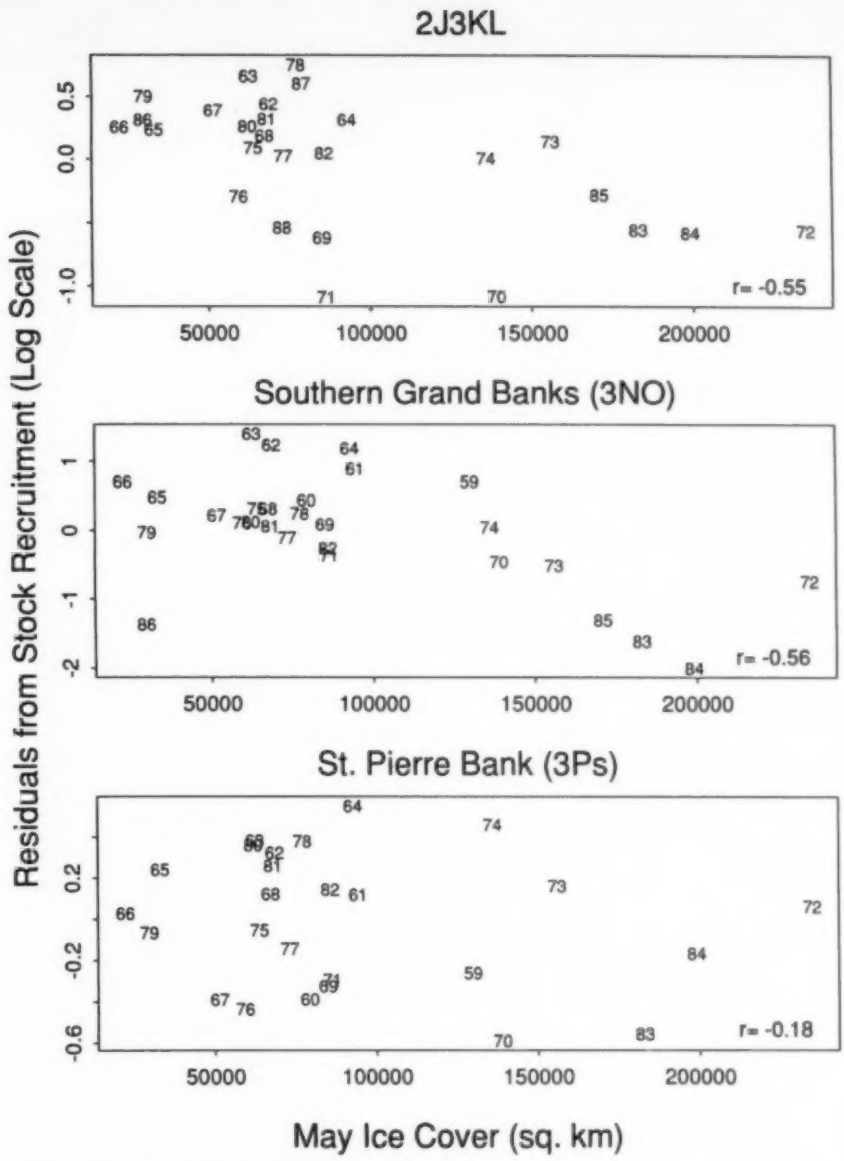


FIG. 7. May ice cover south of 55° latitude and recruitment residuals from a power stock–recruitment curve. The correlation is given in the lower right corner.

examination of historic continuous plankton recorder (CPR) data (Robinson et al. 1973) has shown that salinity is, if anything, negatively correlated with phytoplankton biomass for the Labrador–Newfoundland region (G. Mertz and R.A. Myers, Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada, unpublished data). The correlation of the CPR index of phytoplankton biomass off the Labrador coast with summer surface (0–20 m) salinity at Station 27 from 1961 to 1971 was negative ($r = -0.54$, $p = 0.107$, $n = 10$), as was the correlation with phytoplankton in the Grand Banks region ($r = -0.41$, $p = 0.21$, $n = 11$). Although this analysis cannot rule out the food chain hypothesis in the region, we note that the hypothesis is not consistent with the limited data available.

Larval Freezing Hypothesis

Interannual variability in summer and autumn salinities at Station 27 is strongly related to ice coverage (Myers et al. 1990) and the amount of ice is in turn linked to the ocean temperatures (Petrie et al. 1992). Large quantities of ice are usually accompanied by cold, fresh conditions. Valerio et al. (1992) demonstrated that larval death could result from freezing at temperatures of −1.35°C. Thus, another possible explanation for the apparent relationship between recruitment and salinity is

through freezing of cod larvae.

For this hypothesis to be valid, the near-surface temperature has to be below the freezing temperature for larval fish plasma during the time the larvae are present. In order to investigate this, surface temperatures (Drinkwater and Trites 1986) and the time of peak larval concentrations (Myers et al. 1993) were plotted for several areas (Fig. 6). It appears that this is not a valid hypothesis for any stocks except perhaps the northern components of the 2J3KL stock. In all other stocks, near-surface water very seldom goes below freezing when cod larvae are present.

We continue the analysis by using, as an index of freezing, the sea-ice coverage (in units of square kilometres south of 55° along the Labrador and east coast of Newfoundland. These estimates were derived from two data sets provided by Peterson and Prinsenberg (1990) from 1963 to 1991 and Walsh and Sater (1981) from 1953 to 1984. The area covered by ice was summed over the region and time of interest to arrive at the index. The Peterson and Prinsenberg data were used from 1963 to 1991 and the Walsh and Sater data were used from 1953 to 1962. The two time series, which were calibrated by a linear regression (Fig. 2), are very highly correlated ($r > 0.9$). Our a priori belief was that May ice cover would be most closely connected to recruitment because this is the only month with significant ice cover in which larvae might be present.

A negative relationship between the residuals from a power stock recruitment relationship with ice cover south of 55° latitude (Fig. 5 and 7) supports the hypothesis that ice may be responsible for the larval and possibly 1+ juvenile mortality in 2J3KL. The strongest relationship was found with May ice cover. If mortality is occurring during the larval phase, May is the month during which the larvae would be expected to be most susceptible, since they would have just recently hatched. Consistent with the salinity–recruitment relationship, summer salinities at Station 27 depend upon the spring ice coverage in southern Labrador (Myers et al. 1990). The relationship with salinity during age 1+ is more difficult to understand. Although it is possible that juveniles die from ice contact and freezing during the first winter of life, this seems unlikely given that they have high levels of antifreeze (Goddard et al. 1992).

We repeated the stepwise regression described in Table 1 for 2J3KL cod with the inclusion of May ice cover. May ice cover did not enter significantly into the regression ($p < 0.15$).

There also appeared to be a fairly strong relationship between May ice cover and recruitment in southern Grand Banks (3NO) cod ($r = -0.56$) and a weaker one for St. Pierre Bank (3Ps) cod ($r = -0.18$). It is unlikely that these relationships are directly caused by ice, although they may be due to an indirect relationship between May ice cover and some other environmental variable.

Thus, it appears that larval death by freezing may account for some of the interannual variability in recruitment in 2J3KL cod but it is an unlikely mechanism for the other stocks considered.

Conclusions

A reexamination of the regression relationship between recruitment of 2J3KL cod and upper layer salinity at Station 27 has confirmed that the recruitment trends in the years since it was first published by Sutcliffe et al. (1983) were accurately predicted. This is remarkable, since most previously published relationships between recruitment and environmental variables when reexamined with new data usually (Drinkwater and Myers 1987) but not always (Myers and Drinkwater 1989) break down. A positive relationship between summer salinity and recruitment appears to hold not only for the 2J3KL stock complex, but also for St. Pierre Bank (3Ps) and possibly the southern Grand Banks (3NO) as well. The positive relationship is not an artifact of the method of analysis of commercial catch at age data (VPA) because we confirmed the relationship using research vessel surveys undertaken by Canada, France, and Germany. The salinity–recruitment relationship appears to hold for each of the three regions of the 2J3KL stock complex considered. However, aging errors in the original VPA used by Sutcliffe et al. (1983) could have introduced artificial autocorrelations in the recruitment estimates (Bradford 1991). This may account for the strong relationship they found with salinity at age 2 in their analysis which was not found in recent VPA data or in the research vessel data. The research vessel estimates of recruitment use younger ages to estimate year class than the VPA, and thus will have fewer aging errors.

Although we have used all available data to test the proposed relationships, our analysis has two fundamental limitations. First, the VPA and the research vessel recruitment estimates for the last few years (the 1984–88 year classes) are not independent because the research vessel surveys were used to calibrate the VPA. That both show the same trend in the last few years should not be considered evidence of the validity of the relationship with salinity. Second, using stepwise regression with correlated time series that are themselves autocorrelated can only indicate possible relationships and cannot be interpreted in a confirmatory sense. We believe our results to be reasonably reliable only because of the consistency among the relationships for different regions and surveys.

If the Sutcliffe et al. (1983) regression had been used for predictions during the late 1970's and early 1980's, recruitment would have been greatly overestimated. This is believed to be primarily because of the decline in spawning stock biomass which they did not take into account. This should serve as a caveat to fisheries scientists, that even given a relationship between environmental variables and recruitment, it still may not produce the best predictions. Mindful of this caveat, we predict on the basis of our regression relationship that the 1990 and 1991 year classes of 2J3KL will be low (Fig. 5). Indeed, the 1991 year-class is predicted to be the lowest on record. Similarly low levels of recruitment are predicted for the southern Grand Banks (3NO) and St. Pierre Bank (3Ps) for 1990 and 1991. It should also be noted that under the present SSB levels and the historical salinity range, the regression predicts that recruitment cannot reach the high levels observed in the early to mid-1960's. The regression also provides an alternative to the conventional method for making short-term projections of future recruitment levels (i.e., using the geometric mean of the historical recruitment).

The mechanism underlying the relationship between salinity and recruitment remains elusive. The relationship between ages older than 0+ appears unlikely because there is so little evidence for variable natural mortality unrelated to density for most marine demersal populations (Myers and Cadigan 1993). The original hypothesis suggested by Sutcliffe et al. (1983) was that a nutrient flux from Hudson Strait controlled production on the Labrador Shelf so that high salinities (indicative of more mixing and higher nutrient concentrations) favored biological production. Existing data on phytoplankton standing stocks do

not support this hypothesis because they are negatively correlated with salinity. However, the lack of interannual estimates of phytoplankton and zooplankton production time series limits our ability to adequately test any food chain hypothesis.

We also tested the hypothesis that larval freezing could reduce recruitment. While this could possibly explain the relationship for the more northern stocks, it cannot do so for the remaining stocks, although the relationship with salinity was also high for these stocks. Since the eggs and larvae do not all originate in the north, the freezing hypothesis appears unlikely. Other possible explanations for the salinity recruitment relationship include links through advection-related processes or stability arguments. These are presently being explored.

Acknowledgments

We thank Bruce Atkinson for help with the recruitment data and Gordon Mertz and Ken Frank for many helpful discussions. We thank Ingrid Peterson and John Walsh for providing the ice data. This project is part of the Northern Cod Science Program.

References

BAIRD, J.W., C.A. BISHOP, AND E.F. MURPHY. 1992a. An assessment of the cod stock in NAFO Divisions 2J3KL. NAFO SCR Doc. 92/18 Ser. No. N2063: 77 p.

BAIRD, J.W., C.A. BISHOP, AND E.F. MURPHY. 1992b. An assessment of the cod stock in NAFO Divisions 3NO. NAFO SCR Doc. 92/75.

BAIRD, J.W., C.A. BISHOP, AND R. WELLS. 1986. Estimates of age at maturity for cod in NAFO Divisions 2J+3KLNO. NAFO SCR Doc. 86/122.

BAYLEY, G.V., AND J.M. HAMMERSLEY. 1946. The "effective" number of independent time observations in an autocorrelated time series. J. R. Stat. Soc. 8: 184–197.

BISHOP, C.A. 1984. Estimates of age at maturity for cod in subdivision 3Ps. NAFO SCR Doc. 84/122.

BISHOP, C.A., J.W. BAIRD, AND E.F. MURPHY. 1991. An assessment of the cod stock in NAFO Subdivision 3Ps. CAFSAC Res. Doc. 91/36.

BRADFORD, M.J. 1991. Effects of aging errors on recruitment time series estimated from sequential population analysis. Can. J. Fish. Aquat. Sci. 48: 555–558.

DRINKWATER, K.F., AND R.A. MYERS. 1987. Testing predictions of marine fish and shellfish landings from environmental variables. Can. J. Fish. Aquat. Sci. 44: 1568–1573.

DRINKWATER, K.F., AND R.W. TRITES. 1986. Monthly means of temperature and salinity in the Grand Banks region. Can. Tech. Rep. Fish. Aquat. Sci. 1450: 111 p.

GARRETT, C., AND B. TOULANY. 1981. Variability of the flow through the Strait of Belle Isle. J. Mar. Res. 39: 163–189.

GODDARD, S.V., M.H. KAO, AND G.L. FLETCHER. 1992. Antifreeze production, freeze resistance, and overwintering of juvenile northern Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 49: 516–522.

MYERS, R.A., S.A. AKENHEAD, AND K.F. DRINKWATER. 1990. The influence of Hudson Bay runoff and ice-melt on salinity of the inner Newfoundland shelf. Atmos.-Ocean 28: 241–256.

MYERS, R.A., AND N. CADIGAN. 1991. The reliability of recruitment time series. ICES C.M. 1981/D34.

MYERS, R.A., AND N.G. CADIGAN. 1993. Is juvenile natural mortality in marine demersal fish variable? Can. J. Fish. Aquat. Sci. 50: 1591–1598.

MYERS, R.A., AND K. DRINKWATER. 1989. The influence of Gulf Stream warm core rings on recruitment of fish in the Northwest Atlantic. J. Mar. Res. 47: 635–656.

MYERS, R.A., G. MERTZ, AND C.A. BISHOP. 1993. Cod spawning in relation to physical and biological cycles of the northern Northwest Atlantic. Fish. Oceanogr. 2. (In press)

PETERSON, I.K., AND S.J. PRINSENBERG. 1990. Sea ice fluctuations in the western Labrador Sea (1963–1988). Can. Tech. Rep. Hydrogr. Ocean Sci. 123: iv + 130 p.

PETRIE, B., J.W. LODER, J. LAZIER, AND S.A. AKENHEAD. 1992. Temperature and salinity variability on the eastern Newfoundland Shelf: the residual field. Atmos.-Ocean 29: 14–36.

ROBINSON, G.A., J.M. COLEBROOK, AND G.A. COOPER. 1973. The continuous plankton recorder survey: plankton in the ICNAF area, 1961 to 1971, with special reference to 1971. Int. Comm. Northwest Atl. Fish. Res. Doc. 73/78: 19 p.

SHEPHERD, J.G., AND M.D. NICHOLSON. 1991. Multiplicative modelling of catch-at-age data and its application to catch forecasts. J. Cons. Int. Explor. Mer 47: 284–294.

SUTCLIFFE, W.H., R.H. LOUCKS, K.F. DRINKWATER, AND A.R. COOTE. 1983. Nutrient flux onto the Labrador Shelf from Hudson Strait and its biological consequences. Can. J. Fish. Aquat. Sci. 40: 1692–1701.

VALERIO, P.F., S.V. GODDARD, M.H. KAO, AND G.L. FLETCHER. 1992. Survival of northern Atlantic cod (*Gadus morhua*) eggs and larvae when exposed to ice and low temperature. Can. J. Fish. Aquat. Sci. 49: 2588-2595.

WALSH, J.E., AND J.E. SATER. 1981. Monthly and seasonal variability in the ocean–ice–atmosphere systems of the North Pacific and the North Atlantic. J. Geophys. Res. 86(C8): 7425–7445.

WELLS, R., AND C.A. BISHOP. 1980. Some recent changes in the status of cod stock in Divisions 2J and 3KL. NAFO SCR Doc. 80/VI/101: 15 p.

WELLS, R., M.F. BORGES, AND A. VAZQUEZ. 1984. Status of the cod stock in Division 3M. NAFO SCR Doc. 84/94.

Quantitative Impacts of Lake-Level Stabilization on Material Transfer between Water and Sediment in Newnans Lake, Florida

J.F. Gottgens[1] and T.L. Crisman

Department of Environmental Engineering Sciences, A.P. Black Hall, University of Florida, Gainesville, FL 32611, USA

Gottgens, J.F., and T.L. Crisman. 1993. Quantitative impacts of lake-level stabilization on material transfer between water and sediment in Newnans Lake, Florida. Can. J. Fish. Aquat. Sci. 50: 1610-1616.

Spillways at lake outlets reduce water-level fluctuations but may accelerate sedimentation in the lake. In eutrophic Newnans Lake, Florida, a transect of sedimentary profiles, dated with ^{210}Pb and ^{137}Cs by γ-ray spectroscopy, showed threefold increases in accumulation rates of organic matter, total Kjeldahl nitrogen (TKN), and total phosphorus (TP) 1200 m lakeward of a spillway since its construction in 1967. Concentrations of TKN and TP increased 3.5 and 2.4 times, respectively, in sediments deposited since 1967. These increases were progressively less at stations farther from the spillway. Postspillway accumulation of TP was focused toward the dam whereas recent TKN deposition was similar lakewide. Flocculent sediment (>90% water) accumulated at 1.4 cm/yr. Dams designed to reduce water-level fluctuations may provide short-term benefits for lake access and navigation but in the long-term may accelerate deposition of nutrient-rich detritus, reduce lake volume, cloud the water, alter plant communities, and change lake productivity.

Les déversoirs par où s'épanche le trop-plein des lacs permettent d'aplatir les fluctuations de niveau des eaux, mais ils peuvent accélérer la sédimentation lacustre. Dans le lac eutrophe Newnans de Floride, une série de profils sédimentaires datés par spectroscopie gamma au ^{210}Pb et au ^{137}Ca a montré que la vitesse d'accumulation de matière organique, d'azote de Kjeldahl total (NKT) et de phosphore total (PT) à 1 200 m du déversoir, vers le centre du lac, avait triplé depuis la construction de l'ouvrage, en 1967. Les concentrations de NKT et de PT avaient été multipliées respectivement par 3,5 et par 2,4 dans les sédiments déposés depuis 1967. Ces coefficients de multiplication diminuaient progressivement en raison de l'éloignement du déversoir. L'accumulation de PT après la construction de l'ouvrage augmentait à mesure que l'on s'approchait du barrage, tandis que le dépôt récent de NKT était partout semblable, à la grandeur du lac. Les sédiments floculés (>90 % d'eau) se sont accumulés au rythme de 1,4 cm/an. Les barrages de régularisation des niveaux lacustres peuvent procurer des avantages à court terme pour l'accès aux lacs et la navigation lacustre, mais, à long terme, ils peuvent accélérer le dépôt de détritus riches en éléments nutritifs, réduire le volume d'eau lacustre, troubler l'eau, modifier les associations végétales et perturber la productivité lacustre.

Received July 27, 1992
Accepted March 5, 1993
(JB577)

Reçu le 27 juillet 1992
Accepté le 5 mars 1993

Dams are constructed in lake outlets to reduce water-level fluctuations and optimize use of the lake for irrigation, recreation, and sportfishing. In Florida, with its pronounced wet-dry seasonal cycles, such control structures are common (Davis 1973; Gottgens and Montague 1987). The hydraulic modification produced by these dams, however, may accelerate the accumulation of sediments and nutrient-rich detritus in the lake. This can occur in several ways. First, the design of most spillways eliminates discharge of bottom waters rich in sediments resuspended by wind-induced currents (Sheng and Lick 1979). Outflow is restricted to less turbid surface water flowing over the crest of the spillway. During extended periods of low rainfall when water loss from the lake is confined to evaporation, nutrients and suspended solids are not exported. Second, decreased water-level fluctuations reduce exposure of littoral sediments to air, thus reducing oxidative removal of organic matter and preventing seasonal consolidation of this flocculent material. These effects may be particularly pronounced in shallow lakes with large littoral areas, as in Florida. Third, stabilized lake levels may reduce wetland habitat around the lake because many wetland plants require seasonal fluctuations in water level for growth and reproduction (Gosselink and Turner 1978); the decline of such taxa may alter the wetland's ability to filter nutrients and sediments carried into the lake from the watershed.

Increased accumulation of unconsolidated sediment and nutrient-rich detritus associated with spillways may significantly affect lake ecosystems. Flocculent sediments are easily resuspended in shallow lakes resulting in high turbidity and diminished light penetration in the water column. This may change plant communities and reduce submerged vegetation. Organic turbidity exerts considerable oxygen demand (Hargrave 1969; James 1974) which stresses heterotrophic communities in the lake and promotes nutrient release from the sediments (Mortimer 1971; Theis and McCabe 1978). Finally, flocculent substrate in the littoral zone reduces preferred nesting habitat for many fish, particularly Centrarchidae (Bruno 1984).

In spite of these possible effects, little is known about increases in the rate of sedimentation caused by damming of a lake outlet. Measurement of this rate is essential for quantifying mineral cycling in lakes, including the modeling of nutrient concentrations in water (Vollenweider 1976), and for deter-

[1]Present address: Department of Biology, University of Toledo, Toledo, OH 43606, USA

mining loss of lake volume, sediment budgets, and lake productivity. Material transfer rates can be calculated by determining geochronology in the sediment profile using radioactive decay of ^{210}Pb following burial in sediments (Goldberg 1963). Matching the occurrence of ^{137}Cs in the profile with the onset of widespread atmospheric nuclear testing in the early 1950s (Ritchie et al. 1973) provides an additional age marker. These radiochemical techniques have been used widely to detect changes in sediment accumulation rates caused by urban development in a watershed (Smeltzer and Swain 1985), major storm events (Robbins et al. 1978), and deforestation (Oldfield et al. 1980). They are particularly helpful in gaining insight into past conditions in lakes when a long-term data base is absent. Here, they were applied to quantify the impact of a 1967 spillway on net sedimentation rates in Newnans Lake, Florida. The objective was to determine the temporal effects of a dam on sediment composition and net accumulation rates of organic matter, N, and P.

Site Description

Newnans Lake is a shallow eutrophic lake in central Florida. Maximum and mean depth are 3.6 and 1.5 m, respectively, with a total surface area of 3000 ha. Lacking significant sources of cultural enrichment, water quality is largely determined by regional geology dominated by phosphate-rich sands and clays (Canfield 1981). Since the mid-1950s the lake has maintained high mean levels of total nitrogen (TN) (1.83 $mg \cdot L^{-1}$), total phosphorus (TP) (0.10 $mg \cdot L^{-1}$), chlorophyll *a* (53.8 $mg \cdot m^{-3}$), and low Secchi disk transparency (0.58 m) (Huber et al. 1982). Recent water quality data were similar (Gottgens and Crisman 1991). Net primary productivity was high and variable; measures obtained by oxygen light-dark bottle techniques averaged 0.9 g $C \cdot m^{-2} \cdot d^{-1}$ in 1965-66 (Nordlie 1976), while Crisman (1986) frequently recorded levels in excess of 4.0 g $C \cdot m^{-2} \cdot d^{-1}$ during 1980-82. The lake bottom is covered with a homogeneous layer of highly flocculent organic sediment (Holly 1976; Skoglund 1990). Dense growths of filamentous algae and macrophytes dominated by exotics including water hyacinth (*Eichhornia crassipes*) and hydrilla (*Hydrilla verticillata*) are common. Since 1982 the number of sportfish has declined from 1850 to 227/ha, and black crappie (*Pomoxis nigromaculatus*) has disappeared (Florida Game and Fresh Water Fish Commission 1982-90). Hydraulic residence time (postspillway) has been estimated at 0.8 yr (Gottgens 1992). A largely undeveloped drainage area supplies surface water inflow via several small streams from the north. The lake has a single surface water outlet that has been regulated with a spillway since 1967. The 50-m-long and 1.4-m-high structure consists of removable boards down to the channel bed. Since its construction, average seasonal lake-level fluctuations have been reduced from 110 to 78 cm. This reduction is more pronounced if severe prespillway droughts (1954–56 and 1962–64), during which no water-level records were kept, are included (Skoglund 1990). In this shallow lake, with a very small elevation gradient in the littoral zone, such reduced water-level fluctuations may significantly affect seasonal exposure of littoral sediments and the area of vegetated littoral habitat.

Materials and Methods

Three cores were collected from the lake in the spring of 1989 using a Livingstone piston corer (Livingstone 1955) equipped with 4.1-cm-diameter cellulose butyrate tubes. Core locations were arranged in a transect at 1200, 3000, and 4500 m lakeward of the spillway at water depths of 115, 150, and 150 cm (cores 8, 6, and 1, respectively; Fig. 1). The cores were sectioned in 1 cm intervals, and the samples were stored in plastic at 4°C. Bulk density (milligrams per cubic centimetre) and organic matter content (milligrams per milligram) were determined on 1-cm^3 subsamples for as many as 45 intervals per core. Bulk density was measured by drying of subsamples at 95°C for 24 h, cooling under desiccation, and weighing. Organic matter content was estimated by weight loss on ignition at 550°C for 1 h followed by rehydration with distilled water and redrying at 95°C for 24 h (method 209D, American Public Health Association 1985). Nitrogen was measured as total Kjeldahl nitrogen (TKN) using a Technicon-II semiautomated manifold after digestion following Bremner and Mulvaney (1982) but modified to exclude Se as a catalyst. The digestate was also used for TP determinations. Liberated orthophosphate was determined by the ascorbic acid method (method 424F, American Public Health Association 1985) using a Milton Roy spectrophotometer (model 20) with a 1-cm light path. Between 15 and 20 intervals per core were analyzed for these nutrients. Total carbon (TC) was measured by combustion of the sample at 900°C followed by titration of the evolved CO_2 (Lee and Macalady 1989) using a model 5011 coulometer (Coulometrics). Total organic carbon (TOC) was measured using a combustion temperature of 500°C.

To calculate age-depth relationships in sediment cores, the activity of unsupported ^{210}Pb is estimated by determining total and supported ^{210}Pb activity. Supported ^{210}Pb results from and is maintained by radioactive decay of ^{226}Ra (half-life 1622 yr) in the sediments. Unsupported ^{210}Pb is formed by decay of ^{226}Ra to ^{222}Rn (half-life 3.8 d), which escapes to the atmosphere, decays to ^{210}Pb, and enters the lake via precipitation. Subtracting supported ^{210}Pb from the total measured activity of ^{210}Pb in sediment samples yields the unsupported ^{210}Pb activity, which will decrease with depth in the sediments because of radioactive decay. The age of a sediment layer may then be calculated from its activity of unsupported ^{210}Pb. Because the half-life of ^{210}Pb is only 22.3 yr, this dating technique is restricted to an approximately 150-yr time span.

Activities of ^{210}Pb and ^{137}Cs were measured by direct γ-assay using a coaxial N-type, intrinsic-germanium detector (Princeton Gamma Tech). This type of detector counts over a large range of γ-energies and can be used for simultaneous measurement of supported and unsupported ^{210}Pb, ^{137}Cs, or other γ-emiting radioisotopes of environmental interest (Gäggeler et al. 1976). An outer shield (0.95 cm steel), main shield (10.1 cm lead), and an inner lining (0.05 cm cadmium plus 0.15 cm copper) were used to reduce background radiation at the germanium detector. Samples for isotope analysis were dried at 95°C for 24 h, pulverized by mortar and pestle, weighed, and placed in small plastic Petri dishes (No. 1006, Falcon). Core sections were combined (up to 4 cm) to obtain an adequate sample weight (generally more than 1 g). Petri dishes were sealed with plastic cement and left for a minimum of 14 d to equilibrate radon (^{222}Rn) with radium (^{226}Ra). Counting times varied from 14 to 45 h depending on sample weight; small samples needed longer counting times to minimize uncertainty. For each region of interest, counts were corrected for Compton scattering by subtracting the area below the peak from the total counts. This area was determined by a linear fit through three channel

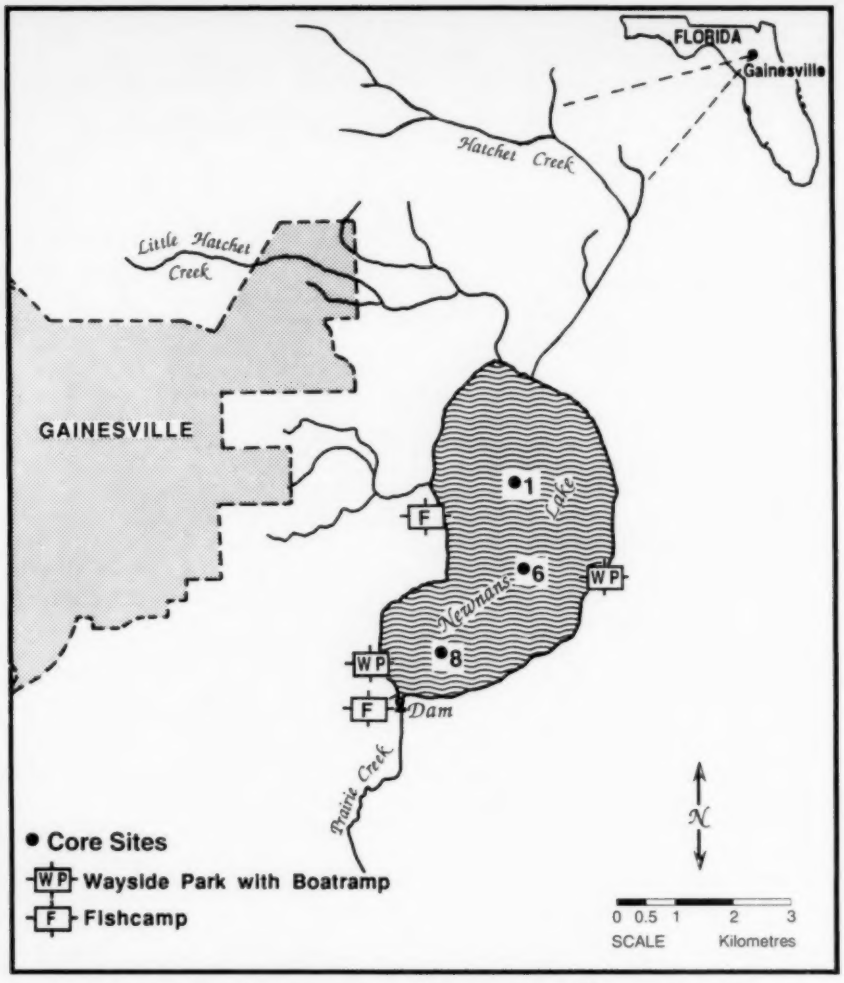


FIG. 1. Location of study area and map of Newnans Lake with core sites indicated.

contents on either side of the region of interest. Blanks were counted for every two samples to determine background from ambient radiation. Standards were run with the same frequency to track efficiency (counts per γ) and to calculate a ^{226}Ra conversion factor (picocuries per count per second) (1 pCi = 37 mBq). Sample spectra were analyzed for activity in the 46.5-keV (^{210}Pb) and 662-keV (^{137}Cs) peaks. Activities at 295 keV (^{214}Po), 352 keV (^{214}Pb), and 609 keV (^{214}Bi) representing uranium series peaks were used to compute supported levels of ^{210}Pb. Calculation of ^{210}Pb dates followed the constant rate of supply model (Goldberg 1963) which is able to quantify changing sediment accumulation rates. This model appears applicable to Florida lakes, particularly because ^{210}Pb residuals match both the known atmospheric flux of this isotope as well as the residuals of nearby cores (Binford and Brenner 1986; Gottgens 1992).

Results of paleolimnological analyses may be presented in units of concentration or as rates of accumulation. Concentration, expressed as a relative measure of sediment composition (e.g., milligrams per gram), is the conventional way of expressing sediment stratigraphy (Shapiro et al. 1971; Pennington 1973; Griffiths and Edmondson 1975). Such data, however, are vulnerable to variations in sedimentation of other components in the profile. Effects of such dilution can be eliminated from the calculations using ratios of components in the sedimentary matrix. Accumulation rates, on the other hand, are normalized to time, thus avoiding the problem of covariance among different sedimentary components. Detailed sediment dating with acceptable analytical precision, necessary for this procedure, can now be done with direct ^{210}Pb low-background γ-assay (Gäggeler et al. 1976). This technique eliminates uncertainties associated with ^{226}Ra-supported activity and provides additional independent data markers in the profile via a simultaneous assay for other γ-emitting isotopes, including ^{137}Cs. Nonetheless, accumulation rate calculations are numerically sensitive to small errors in ^{210}Pb measurements. The uncertainty associated with this may be reduced by averaging accumulation rates over longer periods of time. In this study, both concentration and accumulation rate data are given to provide complementary information about the recent history of the lake.

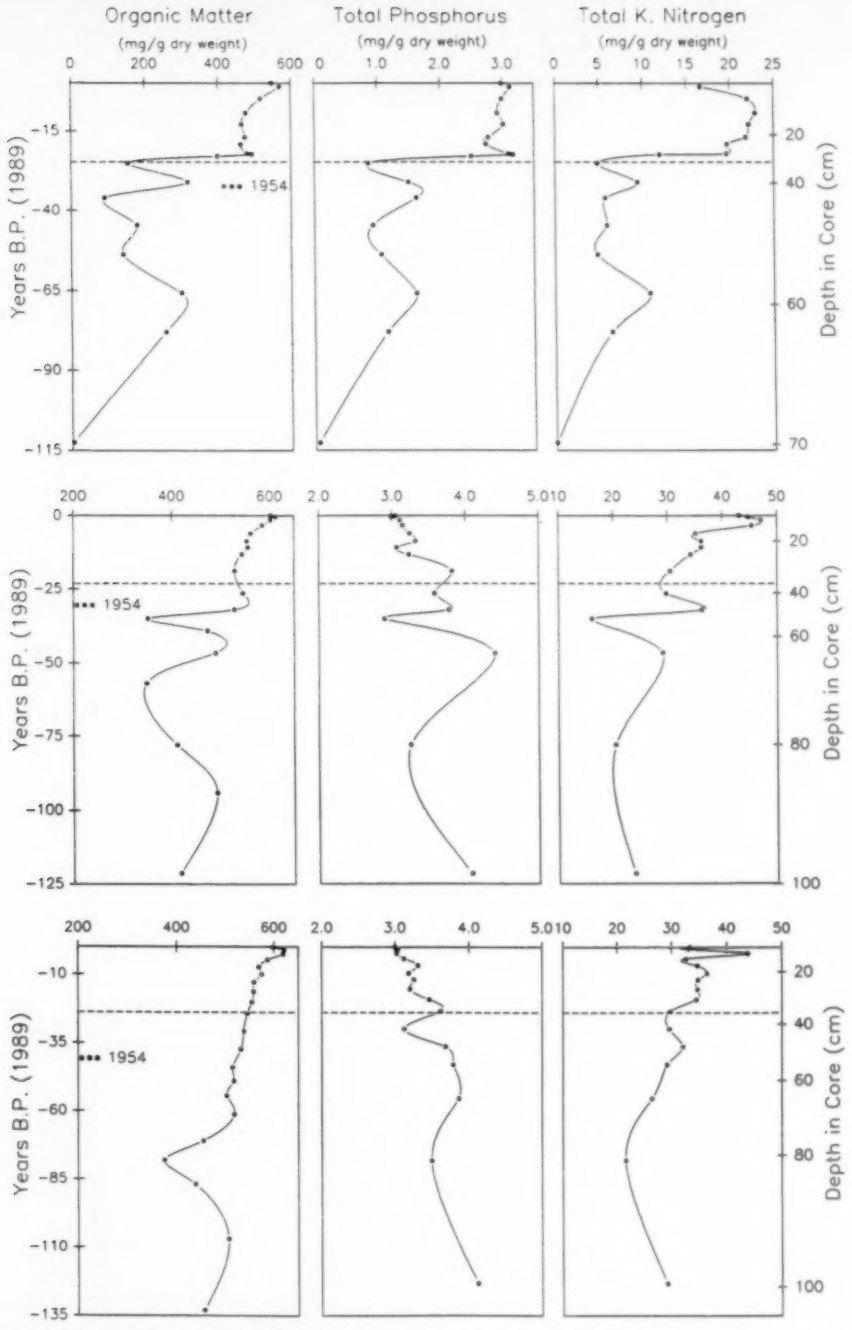


FIG. 2. ^{210}Pb-determined chronologies for core sites 8 (top), 6 (middle), and 1 (bottom), Newnans Lake: profiles for organic matter, TP, and TKN. Spillway installation (broken line) and the first occurrence of the ^{137}Cs marker (asterisks) are indicated.

Results and Discussion

Cores consisted mostly of homogeneous brown material with organic matter content generally greater than 50% and bulk density less than 70 mg/cm^3 throughout the top 30-40 cm (Gottgens 1992). Abrupt increases in the organic matter, TKN, and TP profiles of core 8 at 33 cm depth coincided with a ^{210}Pb-determined sediment age of approximately 1967, i.e., when the lake outflow was dammed (Fig. 2, top). TKN and TP increased 3.5 and 2.4 times, respectively, in sediments deposited 1200 m

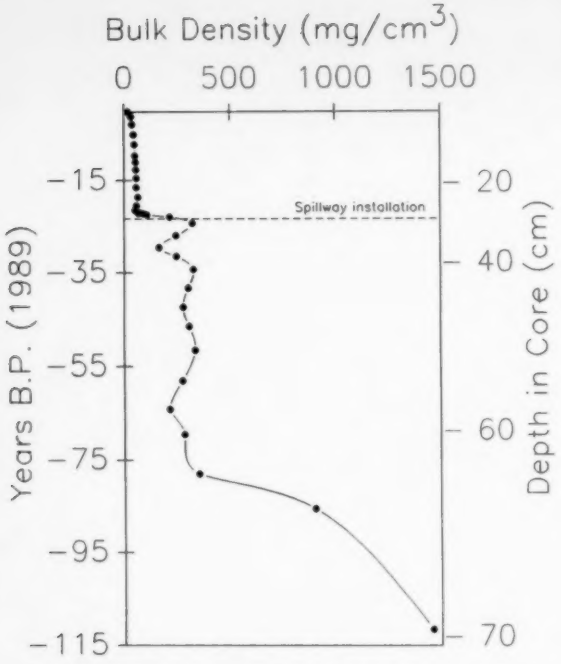


FIG. 3. Bulk density profile for core site 8, Newnans Lake.

lakeward of the spillway since 1967. This signal was progressively less at stations farther from the spillway (Fig. 2). Increased nutrient concentrations at a ^{210}Pb date of approximately 1952–56 matched the driest year (1954) in north Florida's recent history. This may have resulted from reflooding of exposed littoral sediments and subsequent release of nutrients to the water column.

The first occurrence of detectable ^{137}Cs, marking the onset of widespread atmospheric nuclear testing in the early 1950s, matched well with the determined ^{210}Pb chronology (Fig. 2). This agreement is remarkable considering the shallow character of the lake and the flocculence of the bottom substrate that makes these upper sediments vulnerable to physical disturbance. Eutrophic systems such as Newnans Lake, however, accumulate sediment rapidly, so that periodic physical disturbances probably affect only surficial sediments that represent short time intervals. The bulk density profile for core 8 (Fig. 3) demonstrated both the fluid nature of the deposits and the sudden fivefold decrease in bulk density after spillway installation. Bulk density in the top 33 cm of sediment (e.g., 24 yr; hence, a net accumulation of 1.4 cm/yr) was less than 60 mg/cm^3. In most sediment profiles, bulk density increases with depth due to compaction, yet the profile illustrated a significant transition at the time the lake outlet was dammed.

Unconsolidated sediments may be resuspended easily by wind-induced currents in shallow systems such as Newnans Lake. The resulting organic turbidity increases sediment oxygen demand (Bowman and Delfino 1980), particularly in subtropical lakes where high temperatures stimulate bacterial respiration in organic substrate (McDonnell and Hall 1969). In addition, resuspended sediments may contribute significant quantities of nutrients to the overlying water column through desorptive processes or entrained pore fluid (Bengtsson 1975; Cooke et al. 1977). Pollman (1983) reported a more than two-

TABLE 1. TC to TKN to TP ratios (w/w) for cores 8, 6, and 1 pre- and postspillway installation, Newnans Lake.

		Core		
		8	6	1
Post	C:P	82	91	91
Pre	C:P	40	44	43
Post	C:N	13	10	8
re	C:N	7	6	6
Post	N:P	6	10	11
Pre	N:P	6	7	7

fold increase in midlake orthophosphorus concentrations under a moderate wind event (approximately 8.9 m/s) in a central Florida lake with a mean depth and sediment composition similar to Newnans Lake. Pronounced increases in the N and P content of surficial sediments deposited since spillway installation may enhance internal nutrient cycling and therefore change plant communities and lake productivity. For example, using data from Pollman's (1983) sediment dispersion – nutrient release model, resuspension of the upper 1 cm of flocculent sediment at station 8 would cause a 70% increase in the P concentration of the overlying water through entrainment of interstitial water and desorption from suspended sediments.

Carbon to nitrogen to phosphorus ratios (C:N:P) demonstrated both temporal and spatial changes in the composition of the deposits (Table 1). Measurement of TC and TOC demonstrated that almost all sedimentary C is organic in this system (TOC $= 0.996 \times$ TC; SE $= 0.003$). Postspillway deposits were low in P compared with material deposited prior to 1967. This reduction in P content was most pronounced relative to N at stations 6 and 1 (Table 1). Although many factors could be considered in interpreting this difference, including changes in fractionation of sedimentary P and variations in Fe content of the sediments (Engstrom and Wright 1984), such information was not collected for this work. The reduced P content of recent sediments may indicate a change toward P limitation of primary production in the lake.

Spatially within the lake, the amount of P in the sediments relative to C and N was higher in "downstream" areas (e.g., site 8). This may result from rapid entrainment of P and reduced N:P ratios during windy conditions in shallow systems (Hamilton and Mitchell 1988) followed by downstream transport and density-dependent settling of the fine particles with which P is associated (Engstrom and Wright 1984). High C:N ratios in core 8 deposits may reflect a somewhat higher degree of decomposition of bottom material in the lower portion of the lake. The low C:N ratios lakewide (between 6 and 13) imply algal-derived organic matter in the sediment matrix (cf. Wetzel 1983). The contribution of non-Kjeldahl N to this ratio was small in these anaerobic sediments (Reddy and Patrick 1984).

Net accumulation rates of sediment, organic matter, and nutrients increased greatly close to the spillway since its construction (Fig. 4). This increase was progressively smaller at sites farther from the spillway where accumulation rates corresponded well with midlake values reported for lakes of similar trophic state (Binford and Brenner 1986; Deevey et al. 1986). The highest rates were found consistently at the station most distant from significant material inflow in the northern portion of the lake. A 45% higher sedimentation rate at station 8

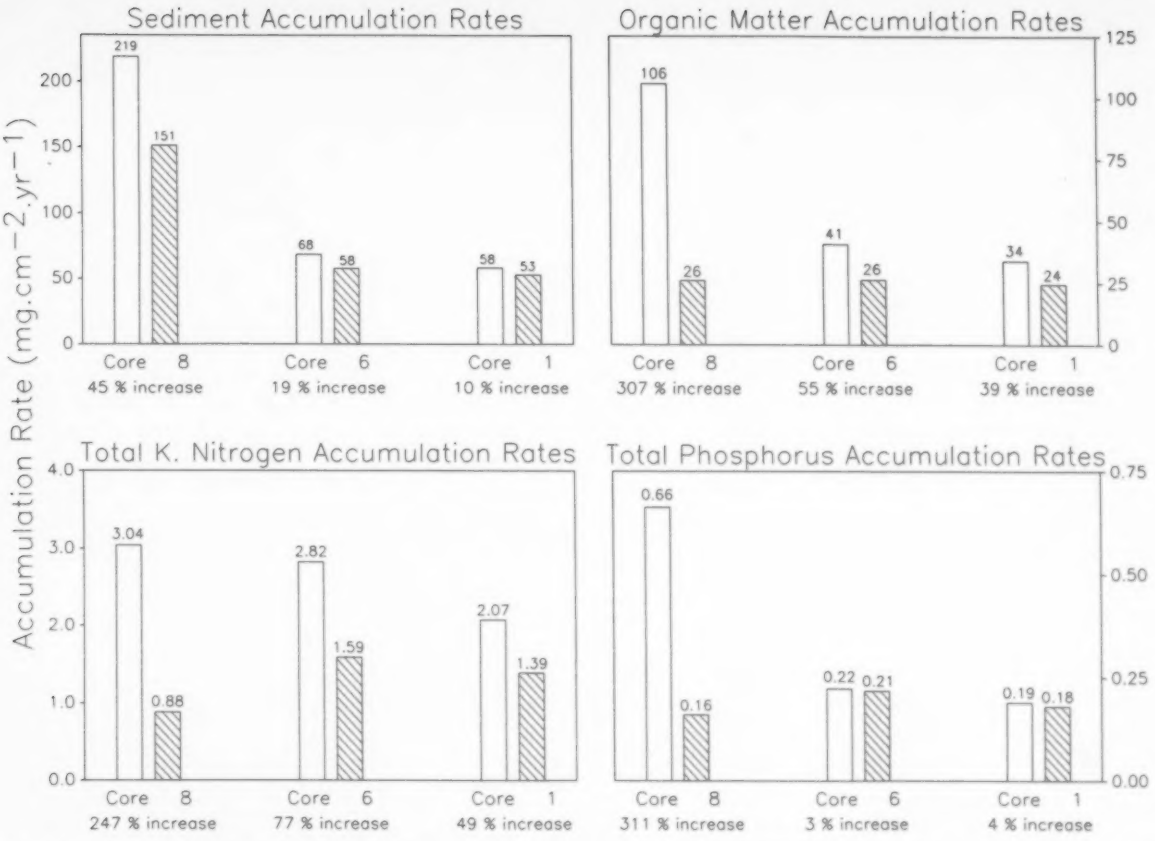


FIG. 4. Net accumulation rates of bulk sediment, organic matter, TKN, and TP for core sites 8, 6, and 1, Newnans Lake. Postspillway rates (open bars) are averaged since 1967; Prespillway rates are averaged from 1900 to 1967. The percent increase since spillway installation is noted.

amounts to an additional 11 cm of material accumulation and a 9% reduction in water depth in this shallow system during the 24 yr since dam construction.

A pronounced increase in the accumulation rate of P at station 8 was observed (3–3.5 times the measured rate at sites 6 and 1) whereas recent N deposition was similar among the three stations. This "focusing" of P in sediments in the lower portion of the lake corresponded to the decreased C:P and N:P ratios at this downstream station. The higher rate of entrainment of P (compared with N) by wind-induced wave action (Maceina and Soballe 1990) probably contributes to P focusing in this shallow, exposed lake. Its short water residence time and obstructed outflow promote horizontal transport and subsequent accumulation close to the dam.

Other factors may have contributed to increased sedimentation, but their effect would have resulted in changed sedimentation rates lakewide, rather than changes concentrated toward the spillway. These factors include herbicide treatment of aquatic macrophytes and added nutrient runoff from the watershed. Between 1972 and 1977, a total of 130 ha (i.e., 4%) of *E. crassipes* and *H. verticillata*. An additional 110 ha was treated in 1988. Analysis of aerial photographs (U.S. Geological Survey 1966-88) did not show significant land-use changes in the basin during the last 22 yr. The number of residences less than 1 km from the Newnans Lake shoreline increased from 16.0 to 19.2 units/km^2 between 1966 and 1988.

In conclusion, the Newnans Lake spillway, intended to stabilize lake stage, changed material transfer between water and sediment. Such changes, operating over a period of decades, can be quantified using radiochemical techniques. A transect of ^{210}Pb-dated profiles perpendicular to the dam at the lake outlet demonstrated a distinct impact from its installation in 1967 on net rate of material accumulation and sediment composition. Net accumulation rate of bulk sediment increased by 45%, resulting in a 9% reduction in water depth; rates for organic matter and nutrients tripled compared with prespillway conditions. Accumulation of P was particularly enhanced close to the spillway compared with other stations. If allowed to persist, increased material transfer between water and sediments may produce loss of lake volume and changes in benthic habitat. The threefold increase in the trapping of nutrients may change plant communities and lake productivity.

Acknowledgements

This work was supported in part by a grant from the St. Johns River Water Management District to J.F.G. D. Leszczynska assisted with the nutrient analyses, and W.E. Bolch and R. Richards consulted on γ-ray spectroscopy. C.L. Montague gave helpful comments on the draft manuscript.

References

AMERICAN PUBLIC HEALTH ASSOCIATION. 1985. Standard methods for the examination of water and wastewater. 16th ed. Washington, D.C. 1273 p.

BENGTSSON, L. 1975. Phosphorus release from a highly eutrophic lake sediment. Verh. Int. Ver. Limnol. 19: 1107–1116.

BINFORD, M.W., AND M. BRENNER. 1986. Dilution of ^{210}Pb by organic sedimentation in lakes of different trophic states, and application to studies of sediment-water interactions. Limnol. Oceanogr. 31: 584–595.

BOWMAN, G.T., AND J.J. DELFINO. 1980. Sediment oxygen demand techniques: a review and comparison of laboratory and in situ systems. Water Res. 14: 491–499.

BREMNER, J.M., AND C.S. MULVANEY. 1982. Nitrogen—total, p. 599-609. In A.L. Page, R.H. Miller, and D.R. Keeney [ed.] Methods of soil analysis. Part II. Chemical and microbiological properties. American Society of Agronomy and The Soil Science Society of America, Madison, Wis.

BRUNO, N.A. 1984. Nest site selection by and spawning season vegetation associations of largemouth bass in Orange Lake, Florida, M.S. thesis, University of Florida, Gainesville, Fla. 94 p.

CANFIELD, D.E. JR. 1981. Chemical and trophic state characteristics of lakes in relation to regional geology. Cooperative Fish and Wildlife Research Unit, University of Florida, Gainesville, Fla. 323 p.

COOKE, G.D., M.R. MCCOMAS, D.W. WALLER, AND R.H. KENNEDY. 1977. The occurrence of internal phosphorus loading in two small eutrophic lakes in northeastern Ohio. Hydrobiologia 56: 129–135.

CRISMAN, T.L. 1986. Algal control through trophic-level interactions: investigations at Lakes Wauburg and Newnans, Florida. Florida Department of Natural Resources, Tallahassee, Fla. 178 p.

DAVIS, J.H. JR. 1973. Establishment of mean high-water lines in Florida lakes. University of Florida Water Resources Research Center 24, Gainesville, Fla. 62 p.

DEEVEY, E.S., M.W. BINFORD, M. BRENNER, AND T.J. WHITMORE. 1986. Sedimentary records of accelerated nutrient loading in Florida lakes. Hydrobiologia 143: 49–53.

ENGSTROM, D.R., AND H.E. WRIGHT, JR. 1984. Chemical stratigraphy of lake sediments as a record of environmental change, p. 11-67. In E.Y. Haworth and J.W.G. Lund [ed.] Lake sediments and environmental history. Leicester University Press, Leicester, U.K.

FLORIDA GAME AND FRESH WATER FISH COMMISSION. 1982–90. Fish management reports for the northeast Florida region. Lake City, Fla.

GÄGGELER, H., H.R. VON GUNTEN, AND U. NYFFELER. 1976. Determination of ^{210}Pb in lake sediments and air samples by direct gamma ray measurement. Earth Planet. Sci. Lett. 33: 119–121.

GOLDBERG, E.D. 1963. Geochronology with ^{210}Pb, p. 121–131. In Radioactive dating. International Atomic Energy Agency, Vienna.

GOSSELINK, J.G., AND P.E. TURNER. 1978. The role of hydrology in freshwater wetland ecosystems, p. 63–78. *In* R.E. Rood, D.F. Whigham, and R.L. Simpson [ed.] Freshwater wetlands: ecological processes and management potential. Academic Press, New York, N.Y.

GOTTGENS, J.F. 1992. Quantitative impacts of lake-level stabilization on sediment and nutrient dynamics: coupling limnology with modeling. Ph.D. thesis, University of Florida, Gainesville, Fla. 197 p.

GOTTGENS, J.F., AND T.L. CRISMAN. 1991. Newnans Lake, Florida: removal of particulate organic matter and nutrients using a short-term partial drawdown. Lake Reserv. Manage. 7: 53–60.

GOTTGENS, J.F., AND C.L. MONTAGUE. 1987. Orange, Lochloosa, and Newnans lakes: a survey and preliminary interpretation of environmental research data. St. Johns River Water Management District, Palatka, Fla. 82 p.

GRIFFITHS, M., AND W.T. EDMONDSON. 1975. Burial of oscillaxanthin in the sediment of Lake Washington. Limnol. Oceanogr. 20: 945–952.

HAMILTON, D.P., AND S.F. MITCHELL. 1988. Effects of wind on nitrogen, phosphorus, and chlorophyll *a* in a shallow New Zealand lake. Verh. Int. Ver. Limnol. 23: 624–628.

HARGRAVE, B.T. 1969. Similarity of oxygen uptake by benthic communities. Limnol. Oceanogr. 14: 801–805.

HOLLY, J.B. 1976. Stratigraphy and sedimentary history of Newnans Lake. M.S. thesis, University of Florida, Gainesville, Fla. 124 p.

HUBER, W.C., P.L. BREZONIK, J.P. HEANEY, R.E. DICKINSON, S.D. PRESTON, D.S. DWORNIK, AND M.A. DEMAIO. 1982. A classification of Florida lakes. Florida Water Resources Research Center 72, Gainesville, Fla.

JAMES, A. 1974. The measurement of benthal respiration. Water Res. 8: 955–959.

LEE, C.M., AND D.L. MACALADY. 1989. Towards a standard method for the measurement of organic carbon in sediments. Int. J. Environ. Anal. Chem. 35: 219–225.

LIVINGSTONE, D.A. 1955. A lightweight piston sampler for lake deposits. Ecology 36: 137–139.

MACEINA, M.J., AND D.M. SOBALLE. 1990. Wind-related limnological variation in Lake Okeechobee, Florida. Lake Reserv. Manage. 6: 93–100.

MCDONNELL, A.J., AND S.D. HALL. 1969. Effects of environmental factors on benthal oxygen uptake. J. Water Pollut. Control Fed. 41: R353–R363.

MORTIMER, C.H. 1971. Chemical changes between sediments and water in the Great Lakes — speculations on probable regulatory mechanisms. Limnol. Oceanogr. 16: 387–404.

NORDLIE, F.G. 1976. Plankton communities of three central Florida lakes. Hydrobiologia 48: 65–78.

OLDFIELD, F., P.G. APPLEBY, AND R. THOMPSON. 1980. Palaeoecological studies of lakes in the highlands of Papua New Guinea: I. The chronology of sedimentation. J. Ecol. 68: 457–477.

PENNINGTON, W. 1973. The recent sediments of Windermere. Freshwater Biol. 3: 363–382.

POLLMAN, C.D. 1983. Internal loading in shallow lakes. Ph.D. thesis, University of Florida, Gainesville, Fla. 191 p.

REDDY, K.R., AND W.H. PATRICK. 1984. Nitrogen transformations and loss in flooded soils and sediments. CRC Crit. Rev. Environ. Control 13: 273–309.

RITCHIE, J.C., J.R. MCHENRY, AND A.C. GILL. 1973. Dating recent reservoir sediments. Limnol. Oceanogr. 18: 254–263.

ROBBINS, J.A., D.N. EDGINGTON, AND A.L.W. KEMP. 1978. Comparative ^{210}Pb, ^{137}Cs, and pollen geochronologies of sediments from Lakes Ontario and Erie. Quat. Res. 10: 256–278.

SHAPIRO, J., W.T. EDMONDSON, AND D.E. ALLISON. 1971. Changes in the chemical composition of sediments of Lake Washington, 1958–1970. Limnol. Oceanogr. 16: 437–452.

SHENG, P.Y., AND W. LICK. 1979. The transport and resuspension of sediments in a shallow lake. J. Geophys. Res. 84: 1809–1826.

SKOGLUND, R.R. 1990. Newnans Lake profile. Florida Game and Fresh Water Fish Commission, Lakeland, Fla. 83 p.

SMELTZER, E., AND E.B. SWAIN. 1985. Answering lake management questions with paleolimnology. Proc. Annu. Conf. North Am. Lake Manage. Soc. Virginia 4: 268–274.

THEIS, T.L., AND P.J. MCCABE. 1978. Phosphorus dynamics in hypereutrophic lake sediments. Water Res. 12: 677–685.

U.S. GEOLOGICAL SURVEY. 1966-88. Topographic maps, 7.5 minute series, scale 1:24,000. Gainesville East (29082-F3-TF-024), Micanopy (N2930-W8215/7.5), Orange Heights (2908-F2-TF-024), and Rochelle (N2930-W8207.5/7.5) quadrangles. Reston, Va.

VOLLENWEIDER, R.A. 1976. Advances in defining critical loading levels for phosphorus in lake eutrophication. Mem. Ist. Ital. Idrobiol. Dott Marco Marchi 33: 53–83.

WETZEL, G.R. 1983. Limnology. 2nd ed. Saunders College, Philadelphia, Pa. 767 p.

Relationship between Juvenile Growth and the Onset of Sexual Maturity of Female North Sea Plaice, *Pleuronectes platessa*

A.D. Rijnsdorp

Netherlands Institute for Fisheries Research, P.O. Box 68, 1970 AB IJmuiden, The Netherlands

Rijnsdorp, A.D. 1993. Relationship between juvenile growth and the onset of sexual maturity of female North Sea plaice, *Pleuronectes platessa*. Can. J. Fish. Aquat. Sci. 50: 1617–1631.

The relationship between juvenile growth and the onset of sexual maturation of female North Sea plaice, *Pleuronectes platessa*, is explored on the individual and population level by analysing individual growth curves back-calculated from otoliths of immature and mature fish and by analysing maturity–length ogives of individual cohorts sampled between 1960 and 1990 from commercial landings from three areas in the North Sea. Back-calculated growth rate of females sampled as mature fish was higher than that of immature females up to 3 yr prior to sampling but lower in the last year. The probability of maturation was significantly related to the length reached as well as to the growth rate in previous years. Maturity–length relationships from market samples showed significant differences in slopes and intercepts between age groups, geographical areas, and year classes. Length at maturation (L_{mat}) decreased with age and increased with latitude. The differences in L_{mat} of individual year-classes corresponded to differences in the growth rate and temperature during early life. Both the evidence obtained from the individual and population analysis suggest a 2- to 3-yr maturation time. A model of the juvenile–adult transition process is proposed that provides a consistent interpretation of the observations.

Le rapport entre la croissance chez les jeunes et le début de la maturité sexuelle des plies femelles de la mer du Nord, *Pleuronectes platessa*, est examiné à l'échelon de l'individu et de la population par analyse des courbes individuelles de croissance calculées à rebours à partir des otolithes des poissons immatures et matures et par analyse des courbes de fréquence de la maturité en fonction de la longueur de chaque cohorte échantillonnée entre 1960 et 1990 dans les débarquements commerciaux de trois régions de la mer du Nord. La vitesse de croissance calculée à rebours chez les femelles échantillonnées et considérées comme matures était supérieure à la vitesse de croissance des femelles immatures jusqu'à 3 ans avant l'échantillonnage, mais plus faible dans la dernière année. La probabilité de maturation était reliée de fa;alcon significative à la longueur atteinte de même qu'à la vitesse de croissance au cours de l'année antérieure. Les rapports de la maturité à la longueur dans les échantillons commerciaux ont présenté des écarts significatifs de la valeur de la pente et de l'abscisse à l'origine d'un groupe d'âge à l'autre, d'une région géographique à l'autre et d'une classe d'âge à l'autre. La longueur au début de la maturité (L_{mat}) a diminué avec l'âge et augmenté avec la latitude. Les écarts entre L_{mat} de chaque classe d'âge correspondaient à la différence entre la vitesse de croissance et la température dans la première période de vie. L'analyse de chaque poisson et des populations porte à croire à une durée de maturation de 2 à 3 ans. On propose un modèle de la transition du stade juvénile au stade adulte qui constitue une interprétation cohérente des observations.

Received September 23, 1992
Accepted April 28, 1993
(JB637)

Reçu le 23 septembre 1992
Accepté le 28 avril 1993

North Sea plaice, *Pleuronectes platessa*, has been exploited intensively for more than a century (Masterman 1914; Wimpenny 1953; Bannister 1978). Rijnsdorp (1989) has shown that the length (L_{mat}) and age (A_{mat}) at sexual maturity have changed since the early twentieth century; fish now become mature at a younger age and at a smaller size than previously. This raises the question as to whether the change in sexual maturation reflects a change in the genetical composition of the population or reflects phenotypic plasticity in response to changes in environmental conditions.

Genetical changes in intensively exploited fish populations are not unlikely because fisheries may act as a strong selective force on the rate of maturation (reviews in Miller 1957; Nelson and Soulé 1986; Stokes et al. 1993). However, few studies have thus far yielded conclusive evidence (Ricker 1981; Reznick et al. 1990). The prerequisite that maturation is under genetic control has been proven for a number of teleost species (Kallman and Borkoski 1978; Purdom 1979; McKenzie et al. 1983; Mork et al. 1984).

Changes in length and age at maturation are not necessarily linked to changes in the genetical composition, as it is well established that these represent plastic characters that respond to environmental conditions (Alm 1959; Stearns and Crandall 1984; Stearns and Koella 1986; Godø and Moksness 1987). Maturation is coupled to the physiology of energy acquisition and hormone kinetics (Thorpe 1989). Ample evidence exists that maturation is linked to the rate of growth during the juvenile phase, and it is generally assumed that a fish matures when it has passed some size or age threshold (Nikolskii 1969; Roff 1982, 1991; Rijnsdorp and van Leeuwen 1985; Thorpe 1986). Stearns and Crandall (1984) argued that the maturation threshold would be neither length nor age specific but would follow a trajectory in the length–age space, as has already been shown for other life-history transitions (Policansky 1982, 1983).The trajectory

can be viewed as adaptative with mortality in the juvenile and adult phase and the growth potential as critical factors (Stearns and Crandall 1984; Stearns and Koella 1986; Kozlowski 1992).

A factor complicating the analysis of changes in length at first sexual maturity is that maturation of the ovary already begins well before the actual spawning season (Barr 1963; Lahaye 1972; Deniel 1981; Rijnsdorp 1989). Various authors have even suggested that the process of ovarian ripening spans more than 1 yr (Franz 1910; Yamamoto 1956; Dunn and Tyler 1969; Dunn 1970; Hilge 1976; Burton and Idler 1984). This would imply that growth in early years could also be associated with maturation later in life.

The central objective of this paper is to examine the effects of juvenile growth on the onset of sexual maturity and to derive a hypothesis that quantifies the effects of growth rate in the last completed year before first spawning as well as in earlier years. The approach chosen was descriptive because the relatively high age and large size at maturation (3–5 yr, 30–36 cm: Rijnsdorp 1989) made an experimental approach less feasible. The indirect approach attempts to interpret the variations in maturation (phenotypic plasticity) in response to variations in growth rate variability in maturity patterns is largely driven by environmental variation, seems reasonable (Roff 1991; Policansky 1993).

The phenotypic plasticity in maturation is analysed on both the individual and population levels. On the individual level, maturation is studied in relation to the back-calculated growth histories of females sampled as either mature or immature. First, individual growth histories are analysed for the association between the length attained and for the relative growth rate at subsequent ages. Second, the relative growth rates of mature and immature females are compared in the years prior to sampling. Third, the probability of maturation is studied in relation to the length at sampling and the relative growth rate in the previous 3 yr. On the population level, the variability in the onset of sexual maturity between year classes and geographical areas is analysed in comparison with differences in juvenile growth and temperature.

Although a descriptive study like the present one will probably never allow one to unravel the intricate interactions between growth rate, fish size, and maturation, which will only become known through ingenious experiments, it should yield a consistent interpretation of various observations as well as testable hypotheses about the critical part of the juvenile phase during which environmental factors (growth, temperature) may influence the juvenile–adult transition.

Materials and Methods

Otolith Samples and Growth Back-calculation

The growth history of individual female plaice was studied by back-calculation of the length at age from the pattern of annuli in the otoliths. The evidence for the validity of the method and an analysis of its accuracy and precision are provided in Rijnsdorp et al. (1990).

Four sets of otoliths were used to estimate individual growth curves. Sets A, B, and C are used to compare growth rates of immature and mature females during different years of life. Set

TABLE 1. Growth and maturation characteristics of female plaice of year classes 1963 (otolith set B) and 1969 (otolith set C) in area SB and of year classes 1980–83 (otolith set A) in area GB. The number of immature (imm) and mature (mat) females was recorded in the first-quarter market samples. The lengths refer to the mean back-calculated lengths.

Age group	Length (cm)	imm	mat	%mat	Length (cm)	imm	mat	%mat
	1963				1969			
I	7.3				8.0			
II	14.9				17.1	62	8	11
III	21.7	8	1	11	25.9	134	87	39
IV	27.7	56	41	42	32.9	35	180	84
V	32.5	44	133	75	37.0	18	234	93
VI	35.7	29	313	92	39.7	3	239	99
	1980				1981			
I	7.0	0	0		8.5	0	0	
II	17.4	2	0	0	18.0	0	0	
III	25.5	88	11	11	25.7	128	10	7
IV	31.6	53	44	45	32.0	159	80	34
V	36.1	10	70	81	35.7	18	138	89
VI	35.4	1	52	98		2	106	98
	1982				1983			
I	8.8	0	0		8.1	0	0	
II	18.1	1	0	0	18.5	0	0	
III	26.4	53	5	9	27.1	100	7	7
IV	31.9	43	33	43		58	53	48
V		21	55	72		4	64	94
VI		1	62	98		0	52	100

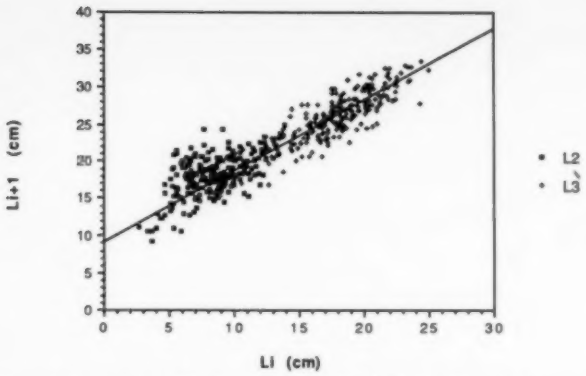


FIG. 1. Scatter plot of the lengths attained by individual females at ages II and III in relation to the length attained in the previous year. The individual growth rate (annual increment) was estimated from the deviance between the observed L_i and the $\hat{L}_i$ predicted from the linear regression line $L_i = a + bL_{i-1}$. Data: otolith set A.

D is used to compare early growth in various areas in the North Sea. Only Set A was established to compare individual growth curves between immature and mature females. The other sets were prepared for other reasons but contained relevant information for investigating the present problem. The geographical areas distinguished are the Southern Bight (SB), German Bight (GB), and Flamborough area (FLAM), which correspond to areas DWK and OG, DB and VB, and FLAM as defined in Rijnsdorp (1989).

Set A comprised 262 otoliths of females of age groups III, IV, V, and VI sampled in January of 1985 and 1986 during two maturity surveys. These maturity surveys were specifically designed to obtain a representative sample of both mature and immature plaice by taking account of differences in spatial distribution (see Rijnsdorp 1989). For each age group, otoliths of both immature and mature females were selected from the samples taken at each station of the lines C, D, and E in GB.

Sets B and C comprised otoliths of year classes 1963 and 1969, respectively, which were sampled at age IV, V, VI, VIII, and X during the spawning period in the southern North Sea (around 53°N, 3°E; area SB) from market samples. For the present purpose, only the data for age groups IV, V and VI were used because these contained both immature and mature females. Year classes 1963 (n = 114) and 1969 (n = 124) were selected because they had shown substantial differences in juvenile growth and maturation (Table 1).

Set D comprised 338 otoliths of mature females sampled for fecundity estimates (Rijnsdorp 1991) in the spawning period between December and March from 1982 to 1985 in SB (n = 152), GB (n = 145), and FLAM (n = 41).

Plaice Samples from Commercial Landings

The landings of plaice by commercial vessels in the Netherlands have been sampled monthly on a routine basis for length, age, weight, sex, and maturity since 1960. These market samples (70–80 fish) are stratified according to geographical area and to the four market size categories in use. From each market category, 15–20 fish are taken at random. Because the Dutch fleet catches more than 50% of the international landings and covers the main distribution area in the North Sea, these data are representative of the entire population (Rijnsdorp 1989). For the analysis of the maturity–length relationships, only market samples from the spawning period (January–March) were analysed. The number of female plaice sampled annually during the spawning period was approximately 200 fish between 1960 and 1967 and >1500 between 1968 and 1990.

Analysis of Individual Growth Rate

The association between the length attained at subsequent ages was studied by determining the correlation matrix between the back-calculated length at successive age, a method first used by Rabinerson (1925, cited in Zamakhaev 1965) and Ford (1928).

The association between the successive growth rates was studied after standardizing growth rate for differences in length at the beginning of the year. Because the annual growth increment in plaice is a decreasing function of fish size (Rijnsdorp et al. 1990), individual growth rate (G_i) can be standardized by calculating the residual of the observed length (L_i) and the predicted length ($\hat{L}_i$) by the regression $L_{i+1} = \alpha + \beta L_i$. Figure 1 gives an example for otolith set A. Growth rate at age 0 was calculated as the difference between the L_1 and the mean L_1.

Statistical Analysis of Maturation

Length at maturation (L_{mat}) was defined as the length at which 50% of the females were mature. Maturation probabilities were studied using logit analysis. Generalized linear models (GLM) were fitted using the NAG statistical package (Baker and Nelder 1978), employing a binominal error distribution and a logit link function (McCullagh and Nelder 1983). The logit link function is analogous to a logit transformation of proportions maturity. Predictor variables were included a priori based on their potential biological role. Statistical significance of the predictor variables were tested with analysis of (co)variance. All variables were tested on their marginal contribution to the model.

Individual level

To analyse whether the growth rates in the subsequent years of the juvenile phase are related to the process of maturation, the relative growth rates in previous years were explicitly included as predictor variables:

$$\text{logit } Y = L + G_{-1} + G_{-2} + G_{-3} + \varepsilon$$

where Y is the state of maturation (mature (1) or immature (2)), L is the length at time of sampling, G_{-1} is the relative growth rate in the last year of completed growth, G_{-2} is the relative growth 2 yr prior to sampling, and G_{-3} is the relative growth 3 yr prior to sampling, and ε is an error term with a binominal distribution. The analysis was carried out with otolith sets A, B, and C. Growth rates were standardized using the regression equation $L_{i+1} = 10.8 + 0.82L_i$, which was estimated from the pooled data of otolith sets A, B, and C for age groups I–VI.

In presenting the results of these analyses, the effect of previous growth (G_i) was expressed as the change in the length at maturation. For L_{mat}, logit $Y = 0$ and the effect of G_i can be calculated from the parameter estimates of the fitted GLM model.

Population level

On the population level, the effect of juvenile growth and temperature on maturation was studied from the variation in maturity–length relationships between cohorts and between geographical areas. Maturity–length relationships were fitted according to the GLM model with a binominal error distribution and a logit link function:

$$\text{logit } Y = X + F1 + F2 + F3 + \varepsilon$$

where Y is the number of mature females, X is the covariable length, $F1$, $F2$, and $F3$ are factors coding for age group, area, and year class, and ε is an error term with a binominal distribution. The number of mature females was analysed per 2-cm size-class. Trippel and Harvey (1991) showed that this method performs well with the type of maturity distributions that were observed in plaice.

The annual variation in juvenile growth was approximated by the length attained at age IV in areas SB, GB, and FLAM separately. Time series information on sea temperature in the nursery area was only available for a coastal station "den Helder" (van der Hoeven 1982), representative of the nursery area of the spawning group in SB.

Results

Individual Growth Patterns

Back-calculated individual growth patterns indicate that a relationship exists between the length attained at successive ages by an individual fish. The matrix of the coefficients of determination of the back-calculated length at different ages for otolith set A is shown in Fig. 2. All values above the arrows are significant at the 5% level. The results for otolith set A are similar to those of otolith sets B and C. The correlation between the length at consecutive ages improves when the fish become older, as shown by the heavy line in Fig. 2. The correlation decreases when length early in life is compared with the length later. For instance, the broken lines in Fig. 2 show that the length at the end of the first year (L_1) has some predictive value for the L_2 but less so beyond L_3. The correlations between L_1 and L_5 and L_6 were not significant.

Individual growth rates can vary significantly, as illustrated by the wide scatter around the regression line in Fig. 1. Individual growth rates at successive ages 0, I, and II appeared to be weakly correlated in all three data sets (Table 2), suggesting that the environmental conditions for growth between consecutive years are variable and unpredictable.

Maturation and Back-calculated Growth of Individual Females

The differences in growth history between females sampled as immature and mature fish during the spawning period show that in all but one of the 48 comparisons (Table 3) the immatures

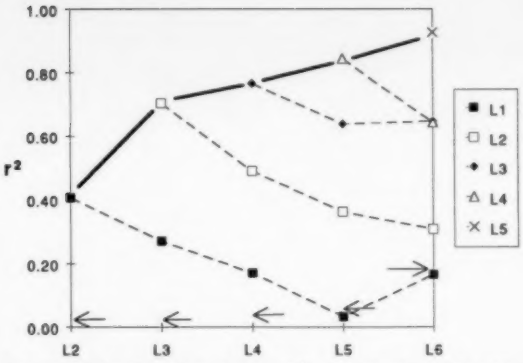


FIG. 2. Coefficients of determination (r^2) of the back-calculated length L_i at different ages i of individual female plaice. The coefficients of determination between the back-calculated lengths at successive ages ($L_2 - L_1$, $L_3 - L_2$, etc.) are shown by the heavy line and between an age group (i = 1–5) and all older age groups (j = 2–6) by the broken lines. Arrows indicate the significance level at $P < 0.05$. Data: otolith set A.

are smaller than matures in the year of sampling as well as in previous years. The single exception is the length attained at age I of females sampled at age V (otolith set A: year-class 1981). Table 3 also shows that the mean length at age of both matures and immatures appears to decline with increasing age at time of sampling.

Maturity–length relationships, logit $Y = L$, were fitted for length at time of sampling as well as for back-calculated length at previous ages (Fig. 3). Following the proportions of mature females by length class backwards from the age of sampling to age I shows that the maturity ogives generally run parallel for the different age groups. Pairwise comparison revealed that none of the slopes differed significantly at the 5% level, except for those at ages I and II for sampling age V, which were significantly flatter than the common slope of ages III–V (F-test, $P < 0.05$).

Because immature females were shorter than mature females during their early life (Table 3), the data of Fig. 3 do not reveal when during the juvenile phase differences in growth rate are related to maturation. Therefore, the growth rate at all ages was compared between females sampled later as immatures and matures. As before, the individual growth rate was standardized by linear regression of L_{i+1} against L_i (i = 1,2) for each otolith set separately.

TABLE 2. Coefficients of determination (r^2) of growth rate at various ages. The growth rate was calculated as the annual length increment (cm) corrected for the length at the start of the growing period. *$P < 0.05$; **$P < 0.01$.

		Age			
Otolith set		0–I	I–II	II–III	III–IV
A	r^2	0.009	0.021*	0.014	0.046*
	n	262	262	182	81
B	r^2	0.000	0.009	0.022*	0.065**
	n	201	201	201	162
C	r^2	0.000	0.034*	0.007	0.060**
	n	191	191	191	151

TABLE 3. Back-calculated lengths at ages I–VI (L_1–L_6 in cm) of immature and mature females sampled at spawning time at different ages.

	Otolith set A						
	L_1	L_2	L_3	L_4	L_5	L_6	n
Year class 1980, sampling age V							
Immature							
Mean	6.6	15.1	22.1	28.2	33.3		6
SD	1.7	3.7	4.2	3.8	4.1		
Mature							
Mean	7.4	18.7	27.3	34.0	38.4		18
SD	2.0	2.8	2.4	2.3	2.1		
Year class 1981, sampling age V							
Immature							
Mean	8.0	17.3	23.8	29.9	34.8		14
SD	2.4	2.6	3.4	3.1	2.5		
Mature							
Mean	7.7	17.6	25.3	31.7	36.2		24
SD	1.8	2.4	2.5	3.3	3.7		
Year class 1981, sampling age IV							
Immature							
Mean	8.3	16.9	24.8	31.8			24
SD	2.2	2.5	2.7	2.9			
Mature							
Mean	9.7	19.8	28.0	33.7			26
SD	2.8	2.8	3.1	3.1			
Year class 1982, sampling age IV							
Immature							
Mean	7.8	16.4	24.0	31.1			27
SD	2.8	3.3	2.8	2.4			
Mature							
Mean	9.4	18.8	26.5	33.0			22
SD	2.3	2.3	2.9	2.2			
Year class 1982, sampling age III							
Immature							
Mean	8.8	18.6	27.5				23
SD	2.4	2.8	3.2				
Mature							
Mean	9.7	19.5	28.9				16
SD	3.0	2.8	2.2				
Year class 1983, sampling age III							
Immature							
Mean	8.0	18.3	27.0				29
SD	1.8	2.6	2.8				
Mature							
Mean	8.4	19.1	27.3				14
SD	1.5	2.0	2.0				

TABLE 3. (*Concluded*)

	Otolith set B						
	L_1	L_2	L_3	L_4	L_5	L_6	n
Year class 1963, sampling age IV							
Immature							
Mean	7.9	16.1	23.2	30.0			28
SD	1.4	1.8	1.6	1.7			
Mature							
Mean	8.9	17.2	24.5	30.3			11
SD	1.6	2.0	1.4	1.7			
Year class 1963, sampling age V							
Immature							
Mean	6.8	13.9	20.2	26.0	31.7		13
SD	1.8	2.0	1.9	1.9	2.3		
Mature							
Mean	7.6	16.0	23.2	29.8	35.5		29
SD	2.5	4.1	4.8	5.4	5.2		
Year class 1963, sampling age VI							
Immature							
Mean	6.9	14.2	20.5	27.0	31.5	35.6	2
SD	0.6	1.7	0.1	0.8	1.4	1.3	
Mature							
Mean	7.3	14.9	22.4	28.5	34.1	38.5	31
SD	1.8	2.3	3.2	3.6	3.6	3.5	

	Otolith set C						
	L_1	L_2	L_3	L_4	L_5	L_6	n
Year class 1969, sampling age IV							
Immature							
Mean	7.5	15.3	24.6	31.9			6
SD	1.4	1.5	3.3	2.3			
Mature							
Mean	9.2	19.2	28.8	35.1			34
SD	3.1	4.4	3.9	2.7			
Year class 1969, sampling age V							
Immature							
Mean	4.6	12.2	20.9	28.6	34.0		4
SD	1.3	1.9	3.4	2.4	3.1		
Mature							
Mean	8.1	17.5	26.8	34.0	38.4		39
SD	2.4	3.4	4.5	3.7	3.0		

Because the analysis yielded similar results, the data of the three otolith sets were pooled. Figure 4 shows that for females sampled at ages IV and V, the differences in growth were statistically significant at ages 0, I, and III and ages I, II, and IV, respectively (two-tailed t-test, $P < 0.05$). Females sampled as mature fish showed a faster growth rate than immatures up to the last year before sampling but a significantly slower growth rate in the last year before sampling. The latter indicates that reproduction is associated with a reduction in somatic growth in the last completed year of growth. The age at which the largest positive difference in previous growth between mature and immature females occurred appeared to shift with sampling age: from age 0 for sampling age III, to age I for sampling age IV, and to ages I and II for sampling age V.

The estimated age of the largest growth difference between fish sampled as mature and immature might be biased by the fact that part of the mature fish will already have been mature for some years. Because females became mature from age III onwards (Table 1), this bias will only affect the older age groups. Therefore, we can expect that the estimated growth superiority of mature females may have been underestimated from age IV onwards, which may explain part of the decline in growth

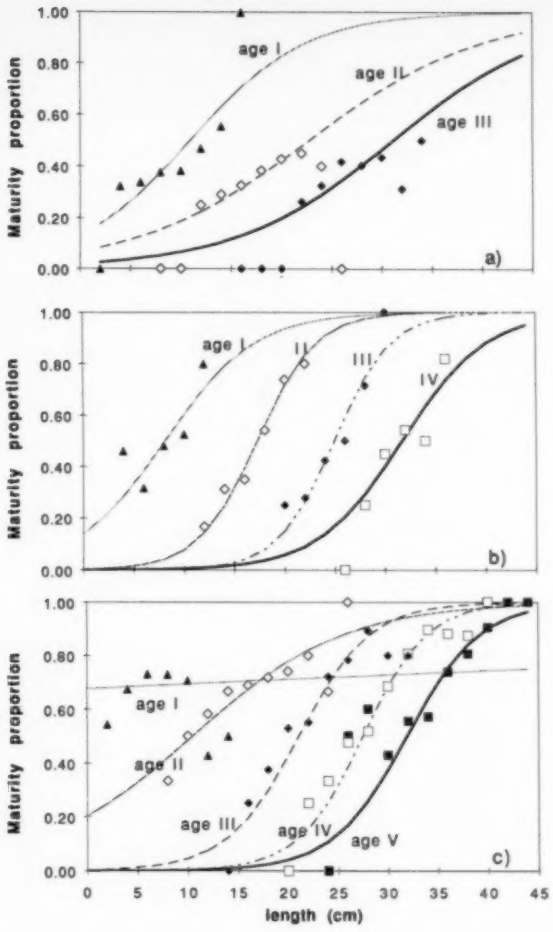


FIG. 3. Maturity proportions in relation to the actual length and to the back-calculated lengths at previous ages (▲, age I; ◇, age II; ◆, age, III; □, age IV; ■, age V) the females sampled at age (a) III, (b) age IV, and (c) age V. The lines show the fitted regressions logit $Y = \alpha + \beta L$.

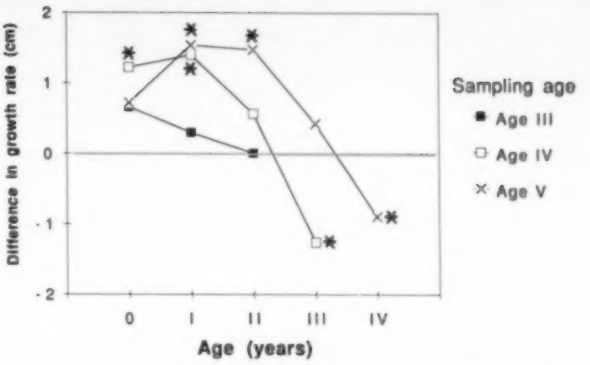


FIG. 4. Difference in previous growth (annual length increment) between female plaice sampled as mature and immature at age III, IV, or V. A positive difference indicates that mature females showed a larger length increment at the age shown on the *x*-axis. The asterisk shows a significant difference (two-tailed *t*-test, $P < 0.05$). Pooled data from otolith sets A, B, and C.

superiority with age. Despite this bias, the results suggest that differences in growth rate in an early phase of life are related to the maturation several years later.

To investigate how growth rate in early life affects the probability of maturation, the individual data from otolith sets A, B, and C were analysed by logit analysis in which the dependent variable reproductive status (immature (0) or mature (1)) was related to the actual length and growth rate during previous years (see Materials and Methods). The mean and standard deviation of the standardized growth rate for the various age groups of the individual year classes are given in Table 4. For the pooled data set the average growth rate is close to zero for all age groups but differs for the individual year classes. Year class 1963 represents a below-average growth rate whereas the growth rates of year classes 1981 and 1982 are in general above average.

Two analyses were carried out: in the first, the effect of previous growth on the probability of maturation was analysed separately for three sampling age groups III, IV, and V; in the second, sampling age groups were combined.

The result of the first analysis is given as the difference in the L_{mat} in females showing a 1-cm higher growth rate at a particular age (Fig. 5). In all three sampling groups, the largest effect of growth rate seems to occur in the last completed year of growth year and 3 yr before sampling. A fast growth rate 3 yr prior to sampling tended to reduce the L_{mat}, thus increasing the probability of maturation, whereas a fast growth rate in the last completed year of growth year tended to increase the L_{mat}. The results, however, were not statistically significant.

Because in the first analysis the three sampling age groups showed a similar pattern, a second analysis was conducted to explore the effect of previous growth rate (G) on maturation irrespective of sampling age. The analysis showed that, besides actual length, growth rate in both the last completed year (G_{-1}) and 3 yr earlier (G_{-3}) was statistically significant (Table 5). The parameter estimates of the GLM model (Table 6) can be used to calculate the effect of the previous growth history on the L_{mat}. The effect of a 1-cm higher growth 1–3 yr prior to sampling on L_{mat}, keeping the growth rate of the other ages at the standard rate, is illustrated in Fig. 6. Fast growth in the last completed year increases L_{mat}, thus decreasing the probability of maturation, whereas fast growth 3 yr before decreases the L_{mat}.

A further extension of the model including the factors year class and age group raised the multicolinearity threefold and removed the significant effect of G_{-3}. Now, length, year class, age group, and G_{-1} showed a significant effect (Table 5). This result is not surprising because by including both length and age as independent variables in the analysis, a proxy for growth was entered. Hence, the older of two similarly sized fish exhibited by definition a slower growth rate. Further, the average growth rate differed between year classes (Table 4), so this factor can also account for part of the variation in previous growth rate.

The analysis of the probability of maturation in relation to previous growth thus clearly indicated that L_{mat} increased with growth rate in the last completed year (G_{-1}). The effect of the growth rate 3 yr prior to sampling (G_{-3}) suggests that the L_{mat} decreases in fast-growing females, although this effect might also be ascribed to a year class or age effect.

TABLE 4. Standardized growth (cm) at ages 0–V or in 1–3 yr prior to sampling of the year classes studied in otolith sets A (year classes 1979–83), B (year class 1963), and C (year class 1969).

Year class	Age						Previous years		
	0	I	II	III	IV	V	–3	-2	–1
1963									
Mean	–0.4	–1.4	–0.9	–0.4	–0.1	–0.4	–1.0	–0.5	–0.2
SD	2.0	2.0	1.6	1.7	1.5	1.3	1.7	1.5	1.4
n	114	114	114	114	75	33	114	114	114
1969									
Mean	0.2	–0.2	1.4	0.8	–0.5	–1.1	0.9	0.8	–0.3
SD	2.7	2.6	2.5	1.6	1.4	0.9	2.3	1.9	1.6
n	124	124	124	124	84	41	124	124	124
1979									
Mean	0.2	–0.1	0.7	0.5	–1.3	–2.0	0.5	–1.3	–2.0
SD	1.4	1.2	1.4	1.6	1.5	1.0	1.6	1.5	1.0
n	12	12	12	12	12	12	12	12	12
1980									
Mean	–1.3	0.9	0.4	–0.1	–0.7	–2.2	0.0	–0.1	–0.9
SD	2.0	2.5	2.3	1.8	1.5	1.7	2.3	1.9	1.5
n	31	31	31	31	31	8	31	31	31
1981									
Mean	0.2	0.2	0.1	0.1	–0.6		–0.1	0.3	–0.1
SD	2.4	2.2	1.8	1.5	1.3		2.1	1.7	1.4
n	87	87	87	87	38		87	87	87
1982									
Mean	0.6	0.1	0.7	0.5			0.2	0.2	1.0
SD	2.7	2.1	1.8	1.4			2.4	1.8	1.6
n	89	89	89	50			89	89	89
1983									
Mean	–0.1	1.0	1.1				–0.1	1.0	1.1
SD	1.7	2.2	1.3				1.7	2.2	1.3
n	43	43	43				43	43	43
Total									
Mean	0.0	–0.2	0.4	0.2	–0.5	–1.1	0.0	0.2	0.0
SD	2.4	2.4	2.1	1.7	1.4	1.3	2.2	1.9	1.6
n	500	500	500	418	240	94	500	500	500

TABLE 5. ANCOVA of maturity in individual females (Y) as a function of body size (L in cm), standardized growth rate (G_{-i} in cm) in previous 3 yr, and the factors year class (YC) and age (A). The analysis employs a binominal error and a logit link function.

	SS	df	MS	F	P
	Run 1: logit $Y = \alpha + \beta L + G_{-1} + G_{-2} + G_{-3}$				
L	107.0	1	107.0	110.5	<0.01
G_{-1}	11.83	1	11.83	12.22	<0.01
G_{-2}	0.338	1	0.338	0.35	ns
G_{-3}	4.321	1	4.321	4.46	<0.05
Multicolinearity	45.91				
Error	479.3	495	0.968		
Total	648.7	499			
	Run 2: logit $Y = \alpha + \beta L + YCLS + A + G_{-1} + G_{-2} + G_{-3}$				
L	15.48	1	15.48	16.83	<0.001
YC	17.19	6	2.86	3.12	<0.001
A	12.61	3	4.20	4.57	<0.001
G_{-1}	9.31	1	9.31	10.12	<0.01
G_{-2}	0.979	1	0.979	1.06	ns
G_{-3}	0.060	1	0.060	0.07	ns
Multicolinearity	146.07				
Error	447.0	486	0.920		
Total	648.7	499			

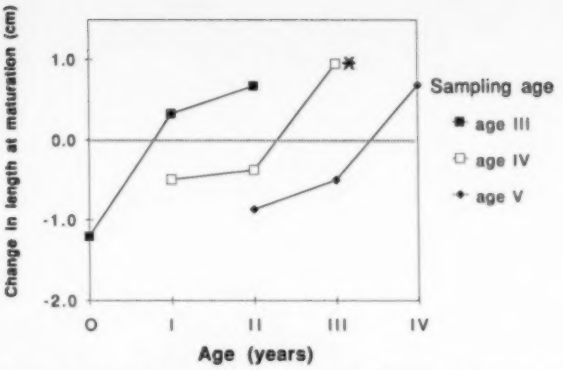


FIG. 5. Change in length at maturation (L_{mat}) of female plaice in relation to a 1-cm higher growth rate at previous ages for females sampled at ages III, IV, and V. The asterisk show the significant difference (two-tailed t-test, $P < 0.01$). Pooled data from otolith sets A, B, and C.

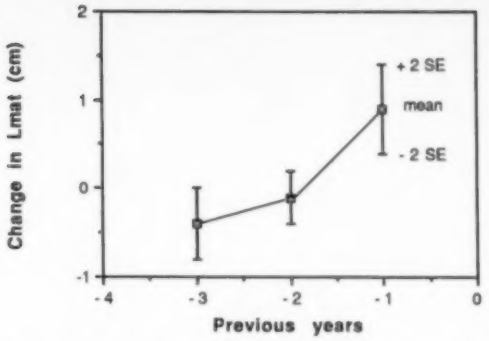


FIG. 6. Change in length at maturation ($L_{mat} \pm 2$ SE) of female plaice in relation to a 1-cm higher growth rate 1, 2, or 3 yr prior to sampling as estimated from the parameter estimates of Table 6.

Maturation and Growth of Cohorts

The variability in the onset of maturation was studied between cohorts and geographical areas from market samples collected between 1960 and 1990. Regression of the number of mature females (Y) per 2-cm size class showed that the maturity–length relationships differed significantly between year classes, areas, and age groups (Table 7). The full model, logit $Y = L + A + AR + YC + L \cdot A + L \cdot YC + L \cdot AR + A \cdot AR + YC \cdot AR$ where L is length, A is age, AR is area, and YC is year class, including the biologically relevant interaction terms, explained 91% of the variance. The significant interaction terms indicate that both the age effect and year class effect differ between areas and that the slope of the maturity ogive differs significantly between age groups, year classes, and areas, respectively. The significant contribution of the covariables age, area, and year class indicates that the intercept differs between these covariables. A substantial part of the explained variation could not be ascribed to single covariables, as indicated by the multicolinearity term.

TABLE 6. Parameter estimates of the GLM model of individual maturity data from Table 5: logit $Y = \alpha + \beta L + G_{-1} + G_{-2} + G_{-3}$ with Y = maturity state of individual females L = body size, and G_{-i} = standardized growth rate in previous 3 yr.

	Parameter	SE
α	−8.244	0.9924
β	0.2698	0.03038
G_{-1}	−0.2406	0.07066
G_{-2}	0.0374	0.06438
G_{-3}	0.1141	0.05535

The significant differences in maturity–length ogives between age groups, both in slope and intercept, indicate that at a particular length the proportion of mature females increases with age and that the predicted L_{mat} decreases with age (Fig. 7). The decrease in L_{mat} with age is illustrated for the three areas from a reduced model including length, age, area, and their interactions but omitting the year class factor. The decrease in L_{mat} with age (Fig. 8) implies that faster growing females mature at a larger

TABLE 7. ANCOVA results of the proportion mature females (Y) of cohorts of female plaice according to the GLM model logit $Y = L \cdot A + AR + YC + L \cdot A + L \cdot YC + L \cdot AR + A \cdot AR + YC \cdot AR$ with L = length (2-cm size clases), A = age (3, 4, 5, 6, 7 yr), AR = area (SB, GB, FLAM), and YC = year class. $L \cdot A$, $L \cdot YC$, $L \cdot AR$, $A \cdot AR$, and $YC \cdot AR$ are the interactions between the main variables. The model employs a binominal error and a logit link function. Data: market samples, 1960–90.

	SS	df	MS	F	P
L	2695	1	2695	1710	<0.01
A	830.0	4	207.5	131.6	<0.01
AR	772.3	2	386.2	245	<0.01
YC	489.2	29	16.87	10.7	<0.01
Multicolinearity	13332				
Error	2322	1473	1.576		
$L \cdot A$	137.1	4	34.28	27.1	<0.01
$L \cdot AR$	31.25	2	15.63	12.4	<0.01
$L \cdot YC$	227.5	29	7.85	6.21	<0.01
$A \cdot AR$	84.8	8	10.60	8.39	<0.01
$YC \cdot AR$	82.9	40	2.073	1.64	<0.01
Multicolinearity	1.4				
Error	1757	1390	1.264		
Total	20440	1509			

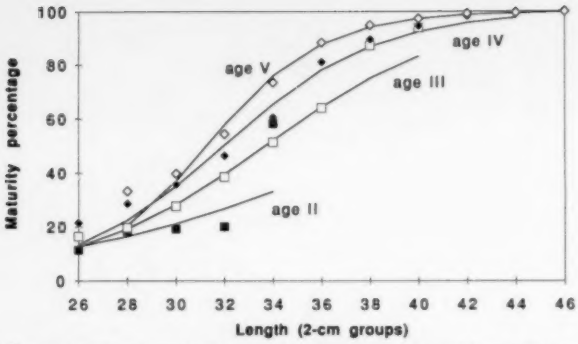


FIG. 7. Maturity–length ogive for females of ages III, IV, V, and VI. The symbols indicate the observed percentage of mature females: ■, age II; □, age III; ◆, age IV; ◇ age V. The lines show the fitted regressions logit $Y = \alpha + \beta L$ for each age group. Data: pooled market sampling data for areas SB, GB and FLAM for the years 1960–1990.

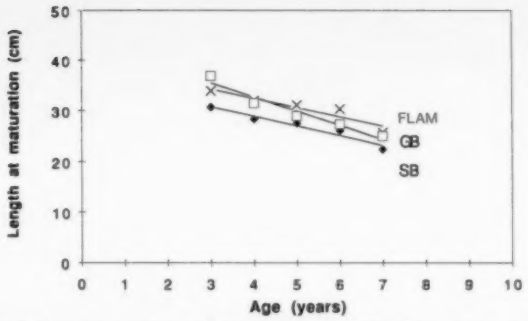


FIG. 8. Linear regressions of the length at maturation (L_{mat}) against age for areas SB (◆), GB (□, and FLAM (×). The symbols show predicted lengths at maturation for ages III–VII according to the full model of Table 7. Data: market sampling, 1960–90.

size and a younger age whereas slower growing ones mature at a smaller length and an older age.

The position of the maturity ogive is determined by its slope and intercept. To simplify the presentation of variations in maturity ogives between year classes and areas, the L_{mat} is used to illustrate the variations. The L_{mat} was calculated for age groups IV and V from the parameter estimates of the full model because at these ages, most females become sexually mature (Table 1). The range of year classes included in the analysis was reduced in areas GB and FLAM owing to the low sampling level in a number of years. The L_{mat} shows substantial variation between year classes in all three areas as illustrated for age group IV (Fig. 9). The coefficient of variation of the fitted L_{mat} was between 5 and 9%.

To test whether the difference in the L_{mat} between year classes is related to the growth rate at an early age, the L_{mat} is correlated with the length attained at age IV (L_4). The L_4 is an approximation of the cumulative growth in the early life. ANCOVA of the total data set according to the model $L_{mat} = L_4 + AR + L4 \cdot AR$ showed

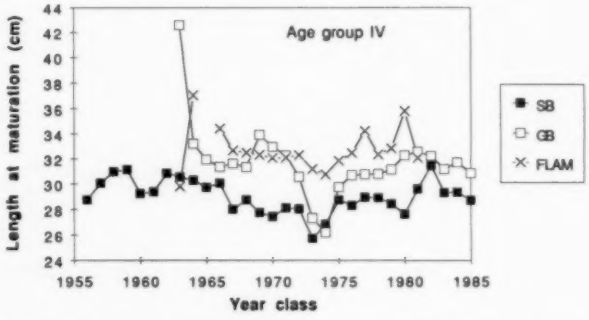


FIG. 9. Variability in the length at maturation (L_{mat}) of 4-yr-old females of year classes 1956–85 in areas SB, GB, and FLAM.

a significant interaction term between L_4 and AR (Table 8), indicating that the slopes of the regression were statistically different between the areas. Forcing a common slope through the

TABLE 8. ANCOVA results of the length at maturation (L_{mat} in cm) at sampling ages IV and V of cohorts of female plaice as a linear function of the length at age IV (L_4 in cm) for three areas in the North Sea (AR = area SB, GB, FLAM) according to the GLM model $L_{mat} = L_4 + AR + AR \cdot L_4$ Data: market samples, 1960–90.

	SS	df	MS	*F*	*P*
		L_{mat} at sampling age IV			
L_4	30.67	1	30.67	9.88	<0.01
AR	126.4	2	113.2	36.5	<0.01
$L_4 \cdot$ AR	28.66	2	14.33	4.62	<0.05
Multicolinearity	30.2				
Error	183.1	59	3.103		
Total	399.0	64			
		L_{mat} at sampling age V			
L	9.98	1	9.98	7.06	<0.01
AR	91.96	2	45.98	32.54	<0.01
$L_4 \cdot$ AR	12.01	2	6.01	4.25	<0.05
Multicolinearity	3.1				
Error	83.36	59	1.413		
Total	200.4	64			

TABLE 9. Results of the F-test for a common slope of the GLM model between the length at maturation and length attained at age IV of cohorts of female plaice sampled in three areas of the North Sea (SB, GB, and FLAM). Data: market samples, 1960–90.

	GB		FLAM	
	Age IV	Age V	Age IV	Age V
SB				
F	2.68	1.31	10.47	9.96
df	1.46	1.46	1.38	1.38
P	ns	ns	<0.01	<0.01
GB				
F			6.364	6.061
df			1.34	1.34
P			<0.05	<0.05

TABLE 10. Parameter estimates of the GLM model of the length at maturation (L_{mat} in cm) against length at age IV (L_4 in cm) of cohorts of female plaice sampled at ages IV and V, according to the model $L_{mat} = (\alpha_{common} + \alpha_{area}) + \beta L_4$. Data: market samples, 1960–90.

L_{mat}	Parameter	SE
Sampling age IV		
α_{common}	50.64	5.917
α_{SB}	Zero aliased	
α_{GB}	2.142	0.584
β	–0.613	0.174
Sampling age V		
α_{common}	52.05	3.89
α_{SB}	Zero aliased	
α_{GB}	0.504	0.384
β	–0.376	0.114

data points showed a significant effect of L_4 on L_{mat}. Pairwise comparison of the regression slopes between the areas showed a significant heterogeneity between areas SB and FLAM and between areas GB and FLAM but not between areas SB and GB (Table 9). The parameters of the GLM model for areas SB and GB are given in Table 10. The regression for area FLAM was not significant. The individual data points and the significant regression lines are shown in Fig. 10 for sampling age IV.

To test whether the geographical difference in the L_{mat} between areas SB, GB, and FLAM is related to the growth rate at an early age, the growth rate in these areas is compared. Prerecruit surveys indicated that the growth rate at age I in GB is significantly lower than in SB (Table 11). No survey data are available for FLAM. Back-calculation of otoliths of mature females of age group VI and older of otolith set D revealed that the standardized growth rate differed between the geographical areas (Fig. 11). Compared with SB, the growth rate was significantly lower at ages I and II in GB (two-tailed *t*-test, $P < 0.05$). The results for FLAM, although not statistically significant, pointed in the same direction (difference at age I, two-tailed *t*-test, $P < 0.10$). The largest difference in standardized growth occurs at age I, which is about 3 yr before first spawning at ages IV and V (Table 1). Therefore, I conclude that the observed latitudinal differences in L_{mat} are associated with a difference in growth rate about 3 yr prior to maturation: fast growth is associated with a reduced L_{mat} 3 yr later.

The effect of temperature on maturation was studied by

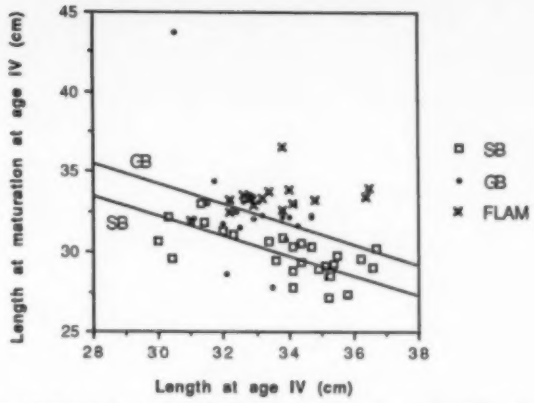


FIG. 10. Relationship between the predicted length at maturation (L_{mat}) of 4-yr-old females of different year classes and the length attained by that year class at age IV (L_4) for areas SB, GB, and FLAM. The significant regression lines shown for areas SB and GB have a common slope. Data: market sampling, 1960-90.

TABLE 11. Average length of age groups 0, I, and II of year classes 1978–86 as observed in the autumn prerecruit surveys carried out in SB and GB (van Beek et al. 1990). *$P < 0.05$.

	Age group		
Area	0	I	II
SB			
Mean	10.1	18.3	25.3
SD	0.6	0.9	1.3
GB			
Mean	9.8	16.2	24.0
SD	0.7	1.3	2.1
Mean difference	0.24	2.06	1.26
t-value	0.78	3.99*	1.52

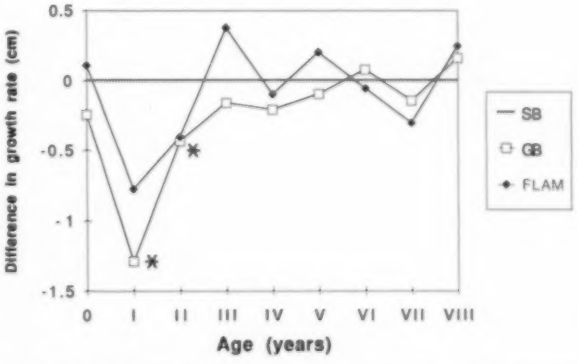


FIG. 11. Differences in growth rate at ages 0–VIII of female plaice in areas GB and FLAM relative to the growth rate in area SB, which is shown by the wavy horizontal line. The asterisk indicates the age groups for which the growth rate differed significantly from area SB ($P < 0.05$). Data: otolith set D.

comparing with the annual differences in L_{mat} of 4 yr-olds in area SB with the annual differences in sea temperature on the nursery grounds in the year prior to sampling. Table 12 shows that only the temperature in the third quarter showed a significant negative relationship with L_{mat}; the correlations in the other quarters were all insignificant.

TABLE 12. Results of the regression analysis of the length at maturation (L_{mat} in cm) of age IV females against the average temperature (T in °C) in quarters 1–4 of the previous year. Model: $L_{mat} = a + bT$. Data: SB market samples, 1960–90.

Quarter	b	r^2	n	P
1	−0.14	0.023	30	ns
2	−0.13	0.004	30	ns
3	−0.70	0.146	30	<0.05
4	−0.12	0.003	30	ns

Discussion

Cohorts were sampled at successive ages to study their maturation in relation to differences in back-calculated growth. This approach implicitly assumes that there is no selective mortality between successive sampling ages. This assumption is not necessarily correct. It has been often observed (Lee's phenomenon) that lengths of the same age group back-calculated from older fish are smaller than those back-calculated from younger fish, and a similar phenomenon is illustrated in Table 3. This phenomenon may be due to an incorrect back-calculation procedure, sampling bias, or size-selective mortality (Jones 1958; Ricker 1969; Casselman 1987). A full discussion of Lee's phenomenon is beyond the scope of this paper, but because the back-calculation method has been validated for plaice (Rijnsdorp et al. 1990), sampling bias and size-selective mortality are the most likely factors involved. In the present case, sampling bias might well apply to the results for otolith sets B and C. These samples were drawn from market samples and consisted of fish above the minimum landing size only. In set A, otoliths were collected from up to four fish per centimetre-group irrespective of sex or maturity. From these samples, otoliths were selected at random from the immature and mature fractions within the age groups of interest. Although stratified sampling by centimetre-group will lead to overrepresentation of the extremes in size distribution, this is not likely to have substantially affected the mean of the back-calculated lengths. The occurrence of Lee's phenomenon in otolith set A might therefore be due to increased mortality with size.

Size-selective mortality due to fishing is not unlikely. Fishing mortality shows a dome-shaped relationship with age that is determined by the differences in spatial overlap of age groups and fishing effort (Rijnsdorp 1993). The decrease in back-calculated length at ages III and IV compared with the length at sampling (Table 13) suggests that the larger sized fish of these age groups have a higher mortality rate, that corresponds to the peak in fishing mortality in age group III (Rijnsdorp 1993). As the larger fish at ages III and IV have a higher probability of maturation, this may imply that maturing females have a higher mortality rate. Rijnsdorp (1989) observed that the maturity percentages of age group III and IV females in the market sampling data were slightly higher than in survey data. This discrepancy was partly related to discarding of undersized and mainly immature fish but may also be related to size-selective mortality inferred above.

The bias in maturity–length relationships due to size-selective fishing mortality is probably small because recruitment to the fishery appears to be size dependent (Beverton and Holt 1957). This inference is corroborated by the general good correspondence between the maturity–length relationships estimated from market samples and from survey results (Rijnsdorp 1989).

The difference in growth rate of maturing and nonmaturing females (Fig. 4) supports the general concept that growth and reproduction are activities that compete for limited resources (Nikolskii 1969; Ware 1980; Roff 1983), although there is evidence in salmonids that hormonally induced differences in behaviour may be involved as well (Metcalfe et al. 1986; Rowe and Thorpe 1990a). That no such effect was observed in females sampled at age III might be related to the fact that the mature 3-yr-old females were all reproducing for the first time (Table 1). Recruit spawners have to build up energy reserves for reproduction but do not have to recover from a depleted body condition following the previous spawning season (Rijnsdorp and Ibelings 1989). Reproduction will thus have its maximum effect on somatic growth after the first reproductive season.

The observation that fast-growing plaice matured earlier and at a larger size than slow-growing ones appears to be a general pattern in teleosts (Nikolskii 1969; Oosthuizen and Daan 1974; Roff 1982; McKenzie et al. 1983; Godø and Moksness 1987; Bowering and Brodie 1991), although exceptions occur (Stearns

TABLE 13. Change in mean back-calculated length of female plaice sampled at two successive ages: year class 1981 sampled at ages IV and V and year class 1982 sampled at ages III and IV. Data from otolith set A.

	Sampling age i			Sampling age i + 1			t-test		
Age	Mean		n	Mean		n	t-test	df	P
				Year class 1981					
I	9.03	3.01	50	7.81	2.05	38	2.15	86	<0.05
II	18.41	3.46	50	17.49	2.48	38	1.39	86	ns
III	26.46	3.80	50	24.75	2.94	38	2.31	86	<0.05
IV	32.79	3.61	50	31.04	3.33	38	2.33	86	<0.05
V				35.68	3.37	38			
				Year class 1982					
I	9.17	2.74	39	8.52	3.09	49	1.03	86	ns
II	18.97	2.88	39	17.48	3.59	49	2.11	86	<0.05
III	28.07	2.97	39	25.12	3.56	49	4.15	86	<0.05
IV				31.95	2.86	49			

and Crandall 1984). This phenomenon is explained by assuming that a fish matures when it has passed a size or age threshold (Nikolskii 1969; Roff 1982; Rijnsdorp and van Leeuwen 1985; Thorpe 1986).

The maturity analysis using market sampling data showed that there were differences in size threshold, approximated by L_{mat}, between geographical areas (Fig. 8) and between individual cohorts. The variations in L_{mat} of cohorts appeared to be correlated with temperature on the nursery grounds in the third quarter of the previous year (Table 13), suggesting that temperature might influence the maturity size threshold. Within the North Sea, differences in the annual temperature cycle occur. In the western North Sea (FLAM area) the summer temperature reaches a maximum in September of approximately 14–16°C compared with 17°C in the southeastern North Sea. In the eastern North Sea north of 55°30′N, the temperature cycle is more steep with a delayed increase to a maximum of approximately 16–17°C in September (Tomczak and Goedecke 1962). Hence, the latitudinal differences in temperature and L_{mat} are consistent with the observed negative correlation between L_{mat} and the average temperature in the third quarter of the previous growing season. Latitudinal differences in maturation, however, are also consistent with differences in juvenile growth, which decreases with latitude (Fig. 11; Bannister 1978; Rijnsdorp 1989; van Beek et al. 1990). Therefore, the latitudinal differences in maturation may be related to both temperature conditions on the nursery grounds and differences in juvenile growth rate.

A genetical explanation seems less likely because studies of enzyme polymorphism did not provide evidence for genetical differences between the various subgroups of plaice (de Ligny 1969; Purdom et al. 1976). Further, tagging experiments have shown an exchange of individual fish between subgroups (de Veen 1978; ICES 1992), which is probably sufficient for generating a gene flow between substocks but insufficient to break up the confinement of the various substocks of plaice in the North Sea (de Veen 1978).

Accepting that environmental conditions during the juvenile phase affect the onset of sexual maturity, the question is at which part in the annual cycle and during which time period of the juvenile phase?

Macroscopic inspection of the ovaries suggested that the time window for maturation was between May and September or earlier (Rijnsdorp 1989). This implies that the L_{mat} estimated during the spawning period overestimates the maturity size threshold. Otolith back-calculation showed that a fast growth in the last completed year was associated with a higher L_{mat} (Fig. 5). The one-to-one correspondence between growth in the last completed year and L_{mat} suggests that the time window may be located at the end of the spawning period in early spring when the annulus in the otolith is formed (Rijnsdorp et al. 1990). At this point in the annual cycle the number of previtellogenic oocytes is formed (plaice; Barr 1963; Atlantic cod (*Gadus morhua*): Kjesbu et al. 1991). Also in salmonids, spring seems to be a critical period in determining smoltification and maturation (Thorpe 1986, 1989; Rowe and Thorpe 1990b).

Logit analysis of maturity data in relation to individual growth history suggested that the standardized growth rate 3 yr prior to sampling affected the probability of being mature. This effect was removed when age and year class were included in the analysis. Because both age and year class were correlated with growth rate, it remains unresolved which combination of the three factors, or some other underlying factor, actually affected maturation. However, the conclusion that early growth rate affects the onset of maturation several years later in life is supported by the observation that the latitudinal differences in L_{mat} (Fig. 8) are associated with a difference in growth rate at ages I and II (Fig. 11).

Most experiments on the effect of previous growth on the onset of sexual maturity focussed on environmental conditions in the year immediately preceding the first spawning and do not provide direct evidence for the possible effect of environmental conditions earlier in the juvenile phase (Waiwood 1982; Rowe and Thorpe 1990a, 1990b; Svedang 1991). At most they provide evidence that size differences in spring, by definition resulting from a faster growth in earlier years, may be associated with maturation in the following year (Berglund 1992).

Support for the hypothesis that growth rate during the early juvenile phase, or some associated factor, may affect the onset of sexual maturation several years later stems from histological studies (Franz 1910; Yamamoto 1956; Dunn and Tyler 1969; Dunn 1970; Hilge 1976; Burton and Idler 1984). These histological studies indicated that the process of maturation could span a period of 2–3 yr. The question of when in the juvenile phase environmental conditions affect maturation seems an intriguing area for future experimental research.

Thorpe (1986) postulated a "physiological" model of maturation in salmonids in which a fish will mature only if the rate of acquisition of surplus energy during a critical period exceeds a genetically determined threshold. Experimental tests yielded general support for the model (Thorpe 1989) but could not explain all observations (Svedang 1991). This model can be accommodated for plaice for the presumed effect of growth rate during the entire juvenile phase by assuming that maturation will be determined by a size threshold that is affected by some factor that accumulates over the entire juvenile period. This factor may be related to the growth rate in a critical period of the annual cycle in successive years during the juvenile period that will fine-tune the maturation threshold; slow growth early in the juvenile phase will increase the threshold size whereas fast growth will decrease the threshold size. It is shown that the accumulation of lipids affects maturation in salmonids. If the lipid level surpasses a threshold level during a critical period in the annual cycle, an individual fish begins to mature (Rowe et al. 1991). Lipid levels, which clearly differ between immature and mature fish (Rijnsdorp and Ibelings 1989), could be a candidate in plaice.

The juvenile–adult transition in plaice can be visualized by an envelope encompassing the individual maturation points in the length–age space (Fig. 12). The maturation envelope in Fig. 12 was constructed by connecting the estimated length at which 10% ($L10$) and 90% ($L90$) of the females had matured for age groups II–VIII; age is in discrete steps of 1 yr because reproduction occurs annually. The $L10$ and $L90$ values were estimated by a reduced GLM model including only length, age, and their interaction as independent variables, thus pooling the data for areas SB, GB, and FLAM and years 1960–90.

The maturation envelope encompasses the juvenile-adult transition points of individual females in the two-dimensional length-age space. The suggestion of the present study, that the probability of maturation is affected by the previous growth history of up to 3 yr, implies that the envelope changes its shape in response to the realized growth trajectories of the individual fish in the population. Therefore, the envelope shown in Fig. 12 refers to the growth trajectories observed during the present period, which are indicated by the length distributions at

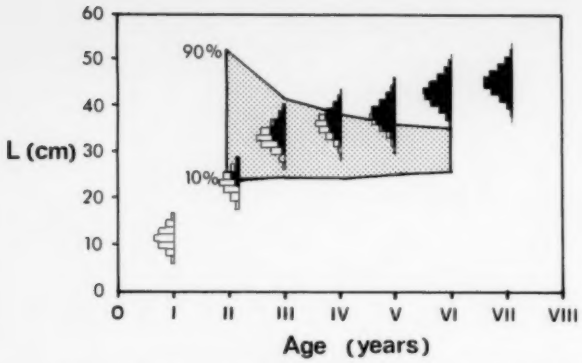


FIG. 12. Maturation envelope (shaded) that encompasses the individual juvenile–adult transitions of female North Sea plaice, representing the present population regarding genetical composition and environmental conditions. The envelope connects the lengths at which 10 and 90% of the females of different age groups become mature. The observed length distributions of immature (open histograms) and mature females (solid histograms) are indicated. Pooled market sampling data, 1960–90, for areas SB, GB, and FLAM.

different ages and the proportions of mature and immature fish. The part of the maturation envelope outside the observed length distributions was extrapolated from the fitted maturity gives for each age group.

The maturation envelope represents the present population of genotypes. Hence, if a change in the genetical make up of the population occurs, with regard to genes affecting the onset of sexual maturity, we may expect a shift in the position of the maturation envelope. In an upcoming paper, the change in position of the maturation envelopes between the present and a historic period will be analysed for the contribution of phenotypic effects and genetic effects due to a change in growth and temperature and to selective fishing.

Conclusions

The present descriptive analysis clearly showed that the probability of maturation was associated with the growth rate in the last completed year. It further provided suggestive evidence for the hypothesis that growth rate, or some associated factor, early in the juvenile phase may affect the probability of maturation 3 yr later. Although this approach can never provide real proof, it illustrates the potential of a descriptive analysis in providing both a consistent interpretation of various field observations and a hypothesis that may be tested experimentally.

Acknowledgements

The contribution of the following persons is gratefully acknowledged: P.I. van Leeuwen, F. Bijlsma, A. de Graaf, H. Verhagen, and P. van der Wateren for their technical assistance, W. Dekker for statistical advice, and Dr. N. Daan and W. Dekker for critically reading an earlier draft of this paper and for many stimulating discussions. The author further acknowledges the helpful comments of an anonymous referree.

References

ALM, G. 1959. Connection between maturity, size and age in fishes. Inst. Freshwater Res. Drottningholm Rep. 40: 5–145.

BAKER, R.J., AND J.A. NELDER. 1978. The GLIM system release. 3. General linear interactive modelling. Numerical Algorithms Group, Oxford, England.

BANNISTER, R.C.A. 1978. Changes in plaice stocks and plaice fisheries in the North Sea. Rapp. P.-v. Réun. Cons. int. Explor. Mer 172: 86–101.

BARR, W.A. 1963. The endocrine control of the sexual cycle in the plaice (*Pleuronectes platessa* L.) I. Cyclical changes in the normal ovary. Gen. Comp. Endocrinol. 3: 197–204.

BERGLUND, I. 1992. Growth and early sexual maturation in Baltic salmon *Salmo salar*. Can. J. Zool. 70: 205–211.

BEVERTON, R.J.H., AND S.J. HOLT. 1957. On the dynamics of exploited fish populations. Fish. Invest. Lond., Ser. 2, Vol. 19: 533 p.

BOWERING, W.R., AND W.B. BRODIE. 1991. Distribution of commercial flatfishes in the Newfoundland–Labrador region of the Canadian Northwest Atlantic and changes in certain biological parameters since exploitation. Neth. J. Sea Res. 27: 407–422.

BURTON, M.P., AND D.R. IDLER. 1984. The reproductive cycle in winter flounder *Pseudopleuronectes americanus* (Walbaum). Can. J. Zool. 62: 2563-2567.

CASSELMAN, J.M. 1987. Determination of age and growth, p. 209–242. In A.H. Weatherley and H.S. Gill [ed.] The biology of fish growth. Academic Press, London.

DE LIGNY, W. 1969. Serological and biochemical studies on fish populations. Oceanogr. Mar. Biol. 7: 411–513.

DENIEL, C. 1981. Les poissons plats (Teleosteens, Pleuronectformes) en Baie de Douarnenez. Ph.D. thesis, Université de Bretagne Occidentale.

DE VEEN, J.F. 1978. On selective tidal transport in the migration of North Sea plaice (*Pleuronectes platessa* L.) and other flatfish species. Neth. J. Sea Res. 12: 115–147.

DUNN, R.S. 1970. Further evidence for a three-year oocyte maturation time in the winter flounder (*Pseudopleuronectes americanus*). J. Fish. Res. Board Can. 27: 957–960.

DUNN, R.S., AND A.V. TYLER. 1969. Aspects of the anatomy of the winter flounder ovary with hypotheses on oocyte maturation time. J. Fish. Res. Board Can. 26: 1943–1947.

FORD, E. 1928. Herring investigations of Plymouth. I. Methods of collection and treatment of data. J. Mar. Biol. Assoc. 15: 237–266.

FRANZ, V. 1910. Die Eiproduktion der Scholle (*Pleuronectes platessa* L.) Wiss. Meeresunters. Neue Folge Band IX: 58–142, 217–224.

GODØ, O.R., AND E. MOKSNESS. 1987. Growth and maturation of Norwegian coastal cod and North-east Arctic cod under different conditions. Fish. Res. 5: 235–242.

HILGE, V. 1976. Ueber die Dauer der Keimzellentwicklung bei Teleosteern. Ber. Dtsch. Wiss. Komm. Meeresforsch. 24: 267–279.

ICES. 1992. Report of the study group on tagging experiments of juvenile plaice. Int. Counc. Explor. Sea C.M. 1992/G:10. (Mimeo.)

JONES, R. 1958. Lee's phenomenon of "apparent change in growth-rate" with particular reference to haddock and plaice. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 1.

KALLMAN, K.D., AND V. BORKOSKI. 1978. A sex-linked gene controlling the onset of sexual maturity in female and male platyfish (*Xiphophorus maculatus*), fecundity in females and adult size in males. Genetics 89: 78–119.

KJESBU, O.S., J. KLUNGSOYR, H. KRYVI, P.R. WITTHAMES, AND M. GREER WALKER. 1991. Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. Can. J. Fish. Aquat. Sci. 48: 2333–2343.

KOZLOWSKI, J. 1992. Optimum allocation of resources to growth and reproduction: implications for age and size at maturity. Trends Ecol. Evol. 7: 15–19.

LAHAYE, J. 1972. Cycles sexuels de quelques poissons plats des cotes bretonnes. Rev. Trav. Inst. Peches Marit. 36: 191–207.

MASTERMAN, A.T. 1914. Report on the plaice fisheries of the North Sea. Fish. Invest. Ser. II, Mar. Fish. G.B. Minist. Agric. Fish Food Vol. 2: 120 p.

MCCULLAGH, P.M., AND J.A. NELDER. 1983. Generalized linear models. Chapman & Hall, London. 261 p.

MCKENZIE, W.D., D. CREWS, K.D. KALLMAN, D. POLICANSKY, AND J.J. SOHN. 1983. Age, weight and the genetics of sexual maturation in the platyfish, *Xiphophorus maculatus*. Copeia 1983: 770–774.

METCALFE, N.B., F.A. HUNTINGFORD, AND J.E. THORPE. 1986. Seasonal changes in feeding motivation of juvenile Atlantic salmon (*Salmo salar*). Can. J. Zool. 64: 2439–2446.

MILLER, R.R. 1957. Have the genetic patterns of fishes been altered by introductions or selective fishing? J. Fish. Res. Board Can. 14: 797–806.

MORK, J., R. GISKEODEGARD, AND G. SUNDNES. 1984. The haemoglobin-polymorphism in Atlantic cod (*Gadus morhua* L.): genotypic differences in somatic growth and maturing age in natural populations. Flod. Rapp. 1: 721–732.

NELSON, K., AND M. SOULÉ. 1986. Genetical conservation of exploited fishes, p. 345–368. *In* N. Ryman and F. Utter [ed.] Population genetics and fishery

management. Washington Sea Grant Program. University of Washington Press, Seattle, Wash.

NIKOLSKII, G.V. 1969. Fish population dynamics. Oliver and Boyd, Edinburgh. 323 p.

OOSTHUIZEN, E., AND N. DAAN. 1974. Egg fecundity and maturity of North Sea cod, *Gadus morhua*. Neth. J. Sea Res. 8: 378–397.

POLICANSKY, D. 1982. Influence of age, size, and temperature on metamorphosis in the starry flounder, *Platichthys stellatus*. Can. J. Fish. Aquat. Sci. 39: 514–517.

POLICANSKY, D. 1983. Size, age and demography of metamorphosis and sexual maturation in fishes. Am. Zool. 23: 57–63.

POLICANSKY, D. 1993. Fishing as a cause of evolution in fishes, p. 000–000. *In* T.K.A. Stokes, R. Law, and J. McGlade [ed.] The exploitation of evolving populations. Springer-Verlag, New York, N.Y. (In press)

PURDOM, C.E. 1979. Genetics of growth and reproduction in Teleosts. Symp. Zool. Soc. Lond. 44: 207–217.

PURDOM, C.E., D. THOMPSON, AND P.R. DANDO. 1976. Genetic analysis of enzyme polymorphisms in plaice (*Pleuronectes platessa* L.). Heredity 37: 193–206.

REZNICK, D., H. BRYGA, AND J.A. ENDLER. 1990. Experimentally induced life-history evolution in a natural population. Nature (Lond.) 346: 357–359.

RICKER, W.E. 1969. Effects of size-selective mortality and sampling bias on estimates of growth, mortality, production and yield. J. Fish. Res. Board Can. 26: 479–541.

RICKER, W.E. 1981. Changes in the average size and average age of Pacific salmon. Can. J. Fish. Aquat. Sci. 38: 1636–1656.

RIJNSDORP, A.D. 1989. Maturation of male and female North Sea plaice (*Pleuronectes platessa* L.). J. Cons. int. Explor. Mer 46: 35–51.

RIJNSDORP, A.D. 1991. Changes in fecundity of female North Sea plaice (*Pleuronectes platessa* L.) between three periods since 1900. ICES J. Mar. Sci. 48: 253–280.

RIJNSDORP, A.D. 1993. Selection differentials and changes in maturation and fecundity in North Sea plaice, p. 000–000. *In* T.K.A. Stokes, R. Law, and J. McGlade [ed.] The exploitation of evolving populations. Springer-Verlag, New York, N.Y. (In press)

RIJNSDORP, A.D., AND B. IBELINGS. 1989. Sexual dimorphism in the energetics of reproduction and growth of North Sea plaice (*Pleuronectes platessa* L.). J. Fish Biol. 35: 401–415.

RIJNSDORP, A.D., AND P.I. VAN LEEUWEN. 1985. Annual variability in age and length at first maturity of North Sea plaice. Int. Coun. Explor. Sea C.M. 1985/G:56.

RIJNSDORP, A.D., P.I. VAN LEEUWEN, AND T.A.M. VISSER. 1990. On the validity and precision of back-calculation of growth from otoliths of the plaice (*Pleuronectes platessa* L.). Fish. Res. (Amst.) 9: 97–117.

ROFF, D.A. 1982. Reproductive strategies in flatfish: a first synthesis. Can. J. Fish. Aquat. Sci. 39: 1686–1698.

ROFF, D.A. 1983. An allocation model of growth and reproduction in fish. Can. J. Fish. Aquat. Sci. 40: 1395–1404.

ROFF, D.A. 1991. The evolution of life-history variation in fishes, with particular reference to flatfishes. Neth. J. Sea Res. 27: 197–207.

ROWE, D.K., AND J.E. THORPE. 1990a. Differences in growth between maturing and nonmaturing male Atlantic salmon (*Salmo salar* L.) parr. J. Fish Biol. 36: 643–658.

ROWE, D.K., AND J.E. THORPE. 1990b. Suppression of maturation in male Atlantic salmon (*Salmon salar* L.) parr by reduction in feeding and growth during spring months. Aquaculture 86: 291–313.

ROWE, D.K., J.E. THORPE, AND A.M. SHANKS. 1991. Role of fat stores in the maturation of male Atlantic salmon (*Salmo salar* L.) parr. Can. J. Fish. Aquat. Sci. 48: 405–413.

STEARNS, S.C., AND R.E. CRANDALL. 1984. Plasticity for age and size at sexual maturity: a life-history response to unavoidable stress, p. 13–33. *In* G.W. Potts and R.J. Wootton [ed.] Fish reproduction: strategies and tactics. Academic Press, London.

STEARNS, S.C., AND J.C. KOELLA. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40: 893–913.

STOKES, T.K.A., R. LAW, AND J. MCGLADE [EDS.] 1993. The exploitation of evolving populations. Springer-Verlag, New York, N.Y. (In press)

SVEDANG, H. 1991. Effects of food quality on maturation rate in Arctic charr, *Salvelinus alpinus* (L.). J. Fish Biol. 39: 495–504.

THORPE, J.E. 1986. Age at first maturity in Atlantic salmon, *Salmo salar*: freshwater period influences and conflicts with smolting. Can. Spec. Publ. Fish. Aquat. Sci. 89: 7–14.

THORPE, J.E. 1989. Developmental variation in salmonid populations. J. Fish Biol. 35(Suppl. A): 295–303.

TOMCZAK, G., AND E. GOEDECKE. 1962. Monatskarten der Temperatur der Nordsee, dargestellt für verschiedene Tiefenhorizonte. Dtsch. Hydrogr. Z. 4(7): 100 p.

TRIPPEL, E.A., AND H.H. HARVEY. 1991. Comparison of methods used to estimate age and length of fishes at sexual maturity using populations of white sucker (*Catostomus commersoni*). Can. J. Fish. Aquat. Sci. 48: 1446-1459.

VAN BEEK, F.A., A.D. RIJNSDORP, AND R. DE CLERCK. 1990. Monitoring juvenile stocks of flatfish in the Wadden Sea and the coastal areas of the southeastern North Sea. Helgol. Meeresunters. 43: 461–477.

VAN DER HOEVEN, P.C.T. 1982. Observations of surface water temperature and salinity. Neth. Inst. Fish. Invest. (RIVO): 1860–1981. KNMI WR.82-8. (Mimeo.)

WAIWOOD, K.G. 1982. Growth history and reproduction in Atlantic cod (*Gadus morhua*), p. 206–208. *In* C.J. Richter and L.J.M.T. Goos [ed.] Reproductive physiology of fish. Pudoc, Wageningen, The Netherlands.

WARE, D.M. 1980. Bioenergetics and stock and recruitment. Can. J. Fish. Aquat. Sci. 37: 1012–1024.

WIMPENNY, R.S. 1953. The plaice. Arnold, London. 144 p.

YAMAMOTO, K. 1956. Studies on the formation of fish eggs. I. Annual cycle in the development of ovarian eggs in the flounder, *Liopsetta obscura*. J. Fac. Sci. Hokkaido Univ. Ser. VI. Zool. 12: 362–373.

ZAMAKHAEV, D.F. 1965. On the question of the effect of growth during the first years of a fish's life on its subsequent growth. Fish. Res. Board Can. Transl. Ser. No. 549.

Relationships among Smolt Size, Marine Growth, and Sea Age at Maturity of Atlantic Salmon (*Salmo salar*) in Northern Spain

A.G. Nicieza and F. Braña

Departamento de Biología de Organismos y Sistemas, Unidad de Zoología, Universidad de Oviedo, 33071 Oviedo, Spain

Nicieza, A.G., and F. Braña. 1993. Relationships among smolt size, marine growth, and sea age at maturity of Atlantic salmon (*Salmo salar*) in northern Spain. Can. J. Fish. Aquat. Sci. 50: 1632–1640.

Scale analysis indicated that two-sea-winter (2SW) Atlantic salmon (*Salmo salar*) had a greater growth increment during the first year at sea than 1SW of the same smolt year in the Rivers Narcea and Esva, but no differences were found between sea age-classes in the River Cares. Interannual variation in marine growth was synchronized among rivers. Variation among years was greater than variation between sea age-classes, suggesting that marine growth per se does not determine age at maturity. In the River Narcea, 2SW salmon were larger than 1SW at the end of the first marine period, but differences between sea age-groups were not significant in the other two rivers. Some of this variability could be attributed to differences in size at smolting; for two rivers (Esva and Cares), 1SW salmon had been larger as smolts than 2SW salmon. Length increment during the first marine growth period was inversely correlated with smolt size (age-1 smolts); in spite of that compensatory effect, large smolts tended to maintain their size advantage at the time of formation of the first marine annulus, as indicated by positive correlations between smolt size and length at the first sea winter.

L'analyse des écailles a montré que le croît du saumon de l'Atlantique (*Salmo salar*) dibermarin était supérieur, au cours de la première année en mer, au croît du saumon y ayant passé un hiver, quand l'année de leur smoltification était la même et que ces poissons provenaient des rivières Narcea et Esva. Aucune différence cependant n'a été trouvée entre les classes d'âge en mer des saumons de la rivière Cares. Les variations interannuelles de la croissance en mer étaient synchronisées entre les rivières. Les variations d'une année à l'autre étaient plus grandes que les variations entre les classes d'âge en mer, ce qui porte à croire que la croissance en mer, à elle seule, ne détermine pas l'âge à la maturité. Dans la rivière Narcea, les saumons dibermarins étaient plus gros, à la fin de la première période en mer, que leurs congénères y ayant passé un hiver, mais les différences entre les groupes d'âge en mer n'étaient pas significatives chez les saumons des deux autres rivières. Une partie de la variabilité pourrait être imputable aux différences de taille à la smoltification; dans les deux rivières (Esva et Cares), le saumon ayant passé un hiver en mer avait été plus gros au stade du smolt que le saumon dibermarin. La croissance en longueur au cours de la première période de croissance en mer était inversement proportionnelle à la taille des smolts (smolts d'âge 1); en dépit de cet effet compensateur, les gros smolts tendaient à conserver l'avantage de taille qu'ils détenaient au moment de la formation du premier annulus en mer, comme le montrent les corrélations positives entre la taille des smolts et la longueur au premier hiver en mer.

Received July 17, 1992
Accepted February 16, 1993
(JB562)

Reçu le 17 juillet 1992
Accepté le 16 février 1993

Age at first reproduction is a crucial parameter in the dynamics of salmonid populations. In the context of life-history theory, it influences fitness through generation time, juvenile mortality, and the size dependence of fecundity or social status (Stearns and Koella 1986). In addition, variability in age at maturity is an important consideration in the management of salmonid fisheries because it can result in large differences in individual size that, in turn, may affect the total biomass of a catch (Bilton et al. 1982). Thus, knowledge of the factors determining age at sexual maturity has become of considerable interest in the study of both life-history theory and fisheries management (e.g., Schaffer and Elson 1975; Jonsson et al. 1984; Glebe and Saunders 1986; Randall et al. 1986).

In Atlantic salmon (*Salmo salar*), sea age at maturity has been shown to have some genetic basis (Nævdal et al. 1978; Nævdal 1983; Gjerde 1984; Gjerde and Gjedrem 1984; Ritter et al. 1986; Chadwick et al. 1987; Skilbrei 1989). Environmental factors could also account for some variation in this trait, although the identity and relative importance of such factors remain largely undetermined. For example, it has been suggested that sea age at maturity is influenced both by freshwater and oceanic conditions. In salmonid species, many studies have found that the length of the seawater phase is inversely related to smolt size (Bilton et al. 1982; Peterman 1982; Nævdal 1983; Hyatt and Stockner 1985; Chadwick et al. 1986, 1987; Randall et al. 1986; Ritter et al. 1986; Ward and Slaney 1988). The inverse relationship between growth in early life stages and age at maturity has been described for other species such as salamanders (Semlitsch et al. 1988), frogs (Smith 1987), and lizards (Heulin 1985). However, several studies carried out on anadromous fishes have suggested that growth conditions during early marine life can influence the age at maturity (Peterman 1985; Scarnecchia et al. 1989). It has been observed that marine growth during the first year at sea usually differs between one-sea-winter (1SW) and older (two-sea-winter (2SW) or multi-sea-winter (MSW)) salmon (Allen et al. 1972; Gardner 1976; Saunders et al. 1983; but see Dempson et al. 1986).

In this study, we examine the relationships between fresh-

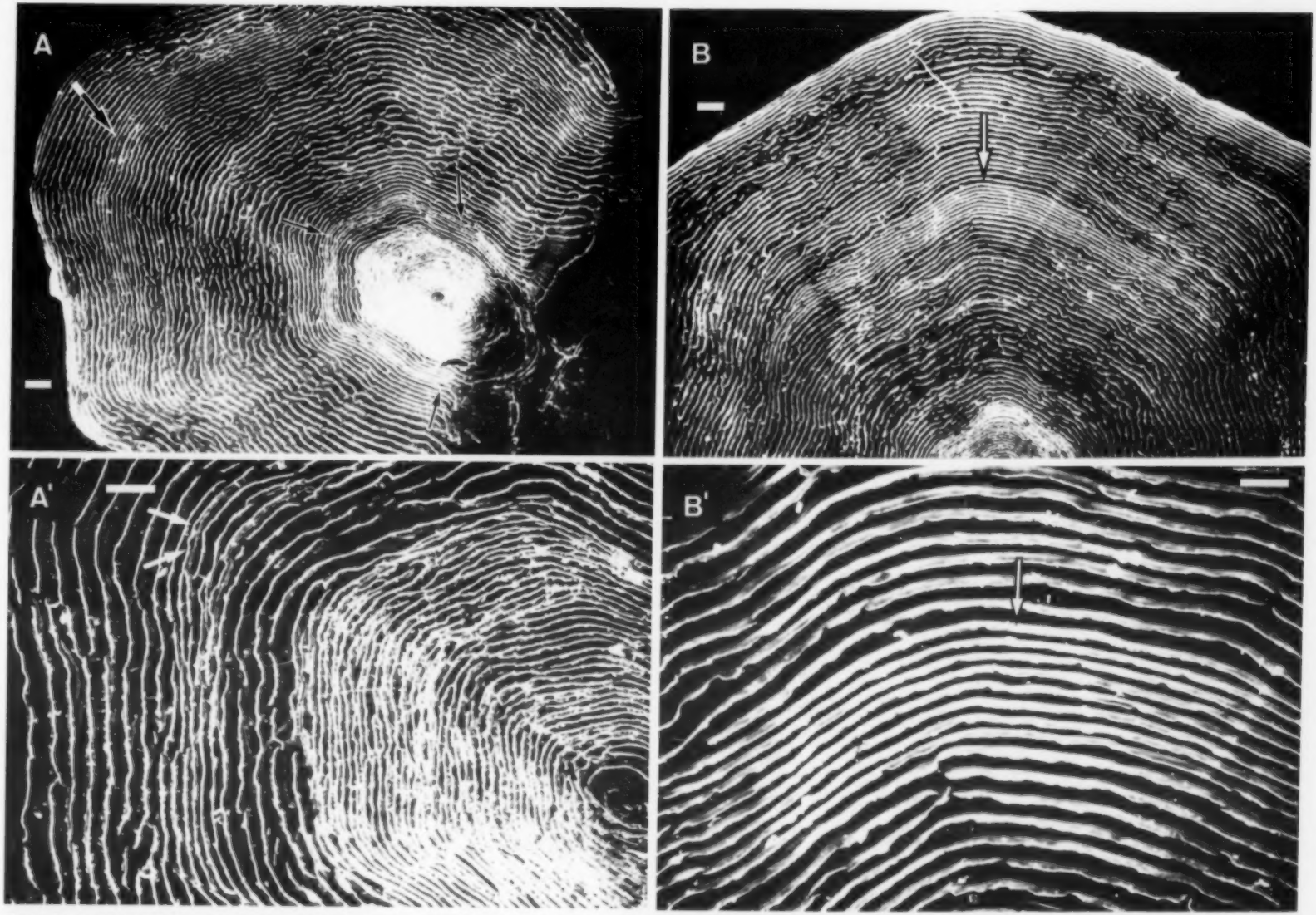


FIG. 1. Scales of adult Atlantic salmon from the River Narcea. Small arrows mark the transition between the freshwater and marine zones. Large arrows mark the sea annulus. Scale bars: A and B, 200 μm; A^1 and B^1, 100 μm.

water and marine growth and the age at maturity for Atlantic salmon populations from three rivers in northern Spain using back-calculation procedures. The primary objective was to determine whether there was any consistent pattern between age at maturity and growth or size at different stages, and if so, to determine the direction of such relationships. This is a critical step in evaluating two alternative predictions that have emerged from the theory of life-history evolution and their applicability to life cycles of anadromous salmonids (Schaffer 1974; Roff 1984; Stearns and Koella 1986): (1) increased growth rate in early (freshwater) life is associated with decreased age at first maturity (Jonsson et al. 1984, Roff 1984) versus (2) growth rate at sea determines age at maturity (Schaffer 1974; Schaffer and Elson 1975; Peterman 1985). To test these predictions, we first looked for differences between fish at different sea age with respect to size at smolting, size at the end of the first sea winter, and growth increment during the first marine year. Second, we compared the frequencies of 1SW and 2SW salmon (1) across different smolt size-classes and (2) among fish with or without evidence of presmolt spring growth in their scales; we assumed that these latter fish were "late" and "early" migrants, respectively (Nicieza and Braña 1993). Fish smolting at different ages or in different years could have different growth rates throughout their life. To remove this source of variation, the main analyses were restricted to river age-1 salmon, and comparisons were made between fish from the same brood year. In addition, we examined the influence of freshwater growth and smolt size on subsequent marine growth.

Materials and Methods

Scale samples were collected from adult Atlantic salmon caught by angling (fishing season: March–July) on the Rivers Narcea (years 1985 and 1987–91), Esva (1987–91), and Cares (1988–91) in Asturias, northern Spain. Stream characteristics have been described elsewhere (Nicieza et al. 1991; Nicieza and Braña 1993). With respect to age composition, these stocks are Type III (see Porter et al. 1986; Nicieza et al. 1990). Most (>70%) of the grilse are male whereas most of the MSW salmon (>60%) are female (Prévost et al. 1991). Fork length was back-calculated to the time of formation of different river and sea winter annuli and time of sea entry for a total of 1655 river age-1 and 262 river age-2 salmon (mainly 1SW and 2SW). The boundary between the freshwater and marine zones was identified as the point on the scale beyond which circuli increase in thickness and spacing and close outward on the scale's posterior zone (Fig. 1A) (see Mathews and Ishida 1989; Holtby et al. 1990); this point usually coincided with a check consisting of two or three thin and closely spaced circuli. Each ocean annulus was identified as an area formed by several relatively fine and closely spaced circuli on the marine zone. Measurements were taken at the point of minimum thickness and spacing of these circuli, which corresponded to the outer edge of the annulus (Fig. 1B) (Mathews and Ishida 1989). Scales having resorbed edges or undiscernible annuli boundaries were not considered for back-calculation. Scale radii were measured to within 0.01 mm using an IMCO-10 system and MIP image analysis software.

Back-calculation was performed using an expression based on the scale proportional hypothesis (Francis 1990) and was applied to a linear model after $\log_{10}$ transformation of fork length and scale radius:

$$L_i = 10^{1.131 + (\log R_i / \log R)(-1.131 + \log L)}$$

where L and R are the fork length (centimetres) and scale radius (millimetres), respectively, at catch and L_i and R_i are the corresponding values at the time of formation of the ith annulus. Other details justifying the selection and measuring of scales and the back-calculation procedure used in this study are given in a previous paper (Nicieza and Braña 1993). River age is denoted as either "1." or "2." indicating the number of winters spent in fresh water; for river age-1 fish, presence or absence of presmolt spring growth is indicated by superscripts, 1^+ and 1^0, respectively. Sea age is denoted as ".1", ".2", or ".X" where X represents all sea ages (.1, .2, and .3) pooled.

For each river data set, we assessed the influence of "year of smolting" and sea age (two-way ANOVA) on smolt size (S), length at the end of the first sea winter (L_{SW1}), and absolute length increment during the first year at sea (marine growth, ΔMG1). We restricted this analysis to age-classes 1.1 and 1.2 because of small sample sizes in other age-classes and considered only smolt years for which at least 25 individuals were available for each age-class. The factorial design was balanced for the Narcea subset (2×3, with random reduction to the minimum cell size: $n = 51$) and because of sampling limitations, proportional between sea age-classes (constant over smolt years) for the Esva and Cares subsets (Esva: 2×4, $n_{1SW} = 25$, $n_{2SW} = 50$; Cares: 2×3, $n_{1SW} = 37$, $n_{2SW} = 50$). In addition, we performed an analysis of covariance of ΔMG1 with sea age and smolt year as class variables and smolt fork length as covariate. Homogeneity of sample variances (Bartlett F-tests), linearity, and homogeneity of slopes were verified prior to the analysis. Because the sex of angled salmon was not available in the catch records, we used samples of 0^+ and 1^+ juvenile salmon taken by electrofishing in the River Pigüeña (the major tributary to the River Narcea) during the winters of 1988–90 to determine whether sexes differed in freshwater growth. The relationships between freshwater and marine growth were examined using a multiple regression analysis with ΔMG1 as the dependent variable and length at the last winter in freshwater (RW1 and RW2 for river age-1 and river age-2 fish, respectively) and the length increment corresponding to the period between the formation of the last river annulus and the smolting check (spring growth, SG) as predictor variables. Stepwise models were obtained for different subsets of fish (age-classes 2.X, 1.X, 1.1, 1.2, and 1^+.X) in each river. The significance level for entry into the model was set at $P = 0.10$. Cases with values of Studentized residuals greater than three were excluded from the regression analyses. In a different approach, 1.1 and 1.2 salmon were classified by 2-cm smolt size-classes, χ^2 tests were carried out to test for independence between smolt size and sea age. This was repeated for "early" (1^0.) and "late" (1^+.) smolts. Statistical analyses were performed with the SPSS/PC+ (version 3.0) and SAS (version 6.02) computer packages.

Results

Smolt Size

There were no significant differences in smolt size between 1.1 and 1.2 salmon from the River Narcea ($F_{1,300} = 2.17$, $P = 0.142$); furthermore, the proportion of both sea age-groups did not change significantly through smolt size categories (Fig. 2; $\chi^2 = 3.40$, df = 5, $P = 0.636$). By contrast,

TABLE 1. Mean lengths (mm) of male and female juvenile Atlantic salmon caught during winter in the River Pigüeña, *t*-tests were used to determine significant differences between sexes.

	Males			Females				
	n	Mean	SD	*n*	Mean	SD	*t*	*P*
Age 0^+								
1988	19	96.5	22.9	35	108.7	21.9	−1.915	0.061
1989	17	97.3	14.9	24	88.7	18.9	1.566	0.125
1990	11	105.9	14.2	10	109.1	16.6	−0.473	0.641
Age 1^+								
1988	20	157.7	11.6	12	157.9	14.3	−0.057	0.954
1989	20	149.9	12.4	20	146.6	13.5	0.806	0.425

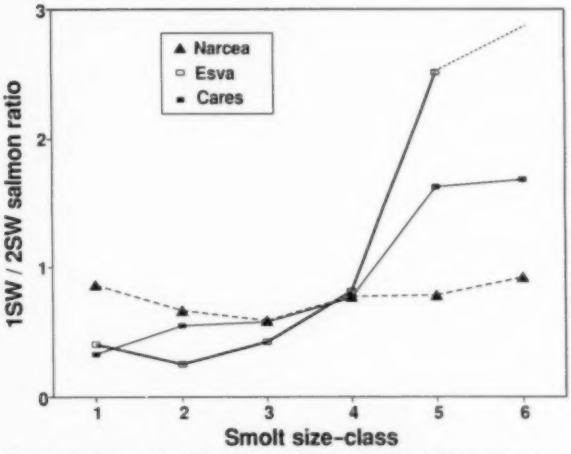


FIG. 2. Relationship between smolt size and the 1SW/2SW salmon ratio (river age 1) in the sample used for back-calculation. Size-classes (cm): 1, 9.0–11.9; 2, 12.0–13.9; 3, 14.0–15.9; 4, 16.0–17.9; 5, 18.0–19.9; 6, >20.0. Results of Spearman rank correlations: Narcea: $r_s = 0.37$, $P > 0.10$; Esva: $r_s = 0.94$, $P < 0.05$; Cares: $r = 1$, $P < 0.001$. In the Esva subset, all fish larger than 20 cm were 1SW salmon.

age 1.1 salmon were characterized by larger smolts than age 1.2 salmon in the Rivers Esva ($F_{1,292} = 8.99$, $P = 0.0029$) and Cares ($F_{1,255} = 8.20$, P 0.0045). In addition, for both rivers the proportion of 1SW fish increased with an increase in smolt size (Cares: $\chi^2 = 19.50$, df = 5, $P = 0.002$; Esva: $\chi^2 = 29.08$, df = 5, $P < 0.001$). These differences were unrelated to the presmolt spring size increment, which was similar among all sea age-groups (ANOVA; Narcea and Esva: $P > 0.5$; Cares: $P = 0.0917$). For each river, the percentage of fish without spring growth was not significantly different among sea age-groups (χ^2 test; df = 1; all rivers, $P > 0.05$). During the period studied, there were significant year effects on smolt size (Narcea: $P < 0.0001$; Esva: $P > 0.1$; Cares: $P < 0.03$) and spring size increment (Narcea and Esva: $P < 0.0001$; Cares: $P > 0.1$). In the samples of juvenile salmon (age classes 0^+ and 1^+) from the River Pigüeña, there were no differences in size between sexes (Table 1).

Marine Growth

In the River Narcea, mean length at the first sea-winter (L_{SW1}) differed between sea age-classes ($F_{1,300} = 12.80$, $P = 0.0004$), and L_{SW1} was generally greater for 2SW salmon. However, neither the River Esva ($F_{1,292} = 1.49$, $P = 0.223$) nor the River Cares ($F_{1,255} = 2.69$, $P = 0.102$) showed significant differences between sea age-classes in L_{SW1} (Fig. 3 and 4). For all three rivers, there was a significant "year" effect on L_{SW1} (two-way ANOVA; Narcea and Esva: $P < 0.0001$; Cares: $P = 0.0178$).

The absolute length increment corresponding to the first year at sea (ΔMG1) was significantly different between sea age-classes for the Rivers Narcea ($F_{1,300} = 5.19$, $P = 0.0234$) and Esva ($F_{1,292} = 6.92$, $P = 0.009$) only, with ΔMG1 generally greater for 2SW salmon (Fig. 4). ANCOVA of ΔMG1 with smolt size as covariate provided similar results (Narcea: $P = 0.0023$; Esva: $P = 0.0712$; Cares: $P = 0.508$). Year effects were significant for all three rivers (Narcea and Cares: $P < 0.0001$; Esva: $P = 0.0023$). For both L_{SW1} and ΔMG1, smolt year × age-class interactions were not significant for any of the rivers. Variations in ΔMG1 were strongly correlated among rivers (Fig. 5A) (Narcea–Cares: $r = 0.933$, $P = 0.0206$; Narcea–Esva: $r = 0.965$, $P = 0.0078$; Esva–Cares: $r = 0.812$, $P = 0.095$). Both L_{SW1} and ΔMG1 were much lower in 1989 than in any other smolt year included in the analysis (they seem to be still lower for the 1990 smolt year, but only data for age 1.1 were available). For all rivers pooled, the $1SW_t/2SW_{t+1}$ salmon ratio was uncorrelated with the mean ΔMG1 (Fig. 5B) (Spearman $r_s = -0.112$, $P > 0.1$, $N = 14$).

Relationships between Freshwater and Marine Growth

To examine the relationships between freshwater and marine growth, ΔMG1 was regressed on fork length at the last river winter (RW1 or RW2) and presmolt spring growth (SG). For all years pooled, size at winter entered the stepwise model ($P < 0.05$) in 16 of 18 (three rivers × six age-classes) subsets. SG entered the regression model only after size at the last river winter had been included in the equation; however, the inclusion of SG resulted in a notable increase in R^2, in most cases greater than 100% (R^2 values ranged from 0.07 to 0.28). Partial regression coefficients were all negative. Maximum condition numbers were smaller than 20, indicating no adverse effects of collinearity on the parameter estimates. Because smolt length is actually formed by length at winter and the subsequent spring growth, multiple R^2 values for the second step can be directly obtained from the regressions of ΔMG1 on smolt size (Table 2). Thus, it seems clear that size at smolting accounted for a proportion of the total variance in ΔMG1 about twice or more than that explained by RW1 or RW2. When age-groups 1.1 and 1.2 were considered separately for each smolt year, the regression of ΔMG1 on smolt size revealed a compensatory

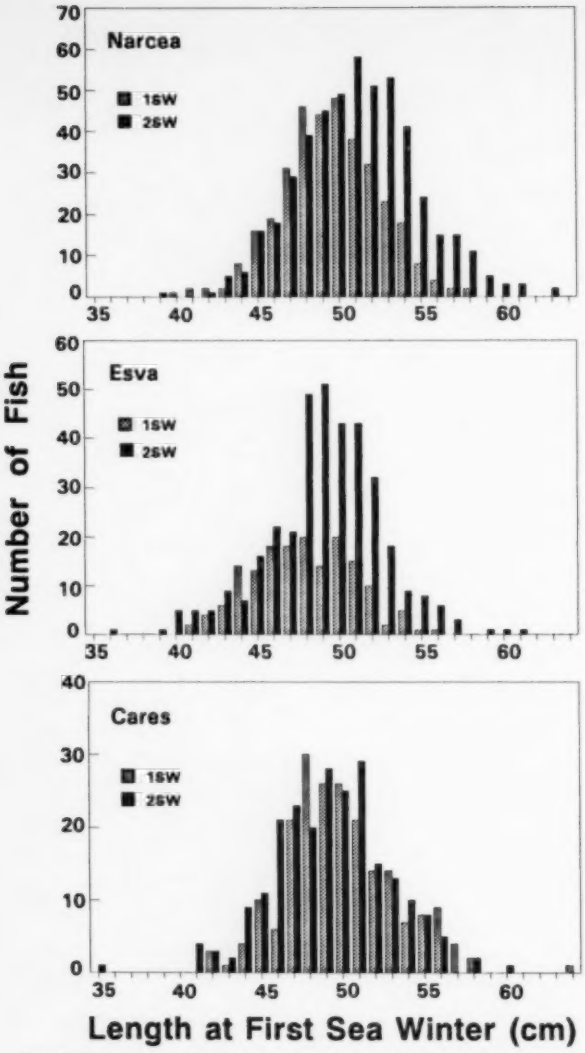


FIG. 3. Frequency distributions of back-calculated length at first sea winter for 1SW and 2SW salmon from the three study rivers (all smolt years pooled).

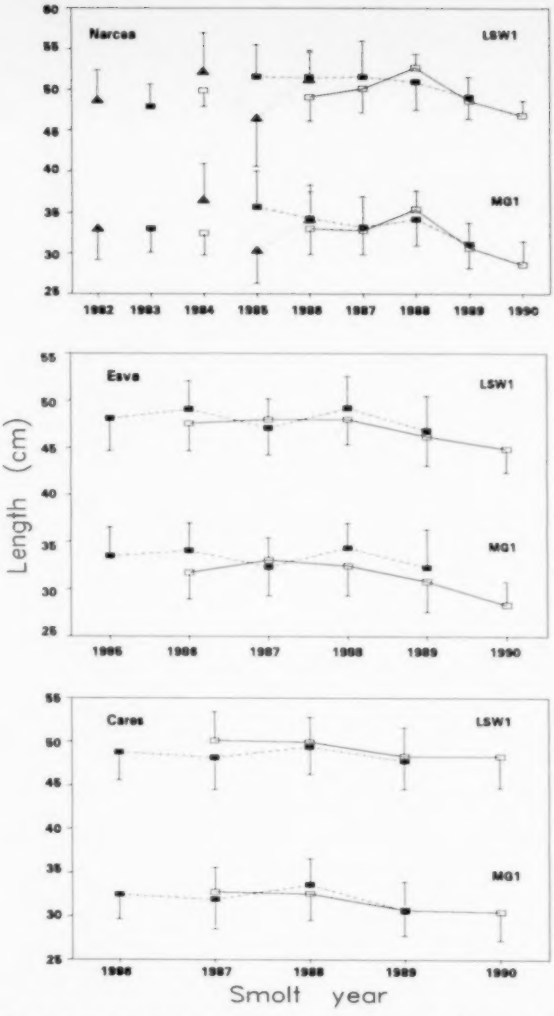


FIG. 4. Annual variation (mean, 1 SD) in length at the first sea winter (L_{SW1}) and marine growth during the first year (ΔMG1) of 1SW (□, 2SW (■, and 3SW (▲, only available for the River Narcea) salmon.

growth pattern, perhaps extensible to the entire cycle (Fig. 6); slopes were negative and differed significantly from zero in most cases (Table 2). Despite that compensatory effect, the regression coefficients for the relationship between S and L_{SW1} were still significant in several subsets (Table 2). Slopes did not differ among years within age-classes (ANCOVA; all river – age-class combinations: $P > 0.15$) or between age-classes (years pooled, all rivers: $P > 0.15$). Lastly, ΔMG1 and SG were negatively correlated in two of six river–age subsets (Narcea, age 1.1: $r = -0.16$, $P = 0.0033$; Cares, age 1.2: $r = -0.17$, $P = 0.0094$).

Discussion

The present study provides empirical support for the prediction that an increased growth rate in early life favors earlier age at first reproduction. In two populations, salmon that spent one winter at sea were larger as age-1 smolts than salmon that spent two winters at sea. Differences in ΔMG1 were statistically significant and consistent over years for salmon from the Rivers Narcea and Esva: 2SW salmon grew faster than 1SW salmon. In addition, growth rate at sea was inversely related to size of smolt; when smolt size effects were removed (ANCOVA), only in one population did 2SW salmon grow faster during their first year at sea than did 1SW salmon. Finally, the relationship between size at the end of the first sea winter and age at maturity was unclear; 2SW were larger in the River Narcea stock but similar in size to 1SW salmon in the other rivers.

Individual size and growth rate at sea have been suggested to influence age at maturity (e.g., Schaffer and Elson 1975; Peterman 1985). For either of these hypotheses to be true, either ΔMG1 or L_{SW1} of 1SW and 2SW salmon should be different, and the direction of these differences should be consistent among stocks and over years within stocks. Moreover, if maturation were triggered at a fixed size or growth rate, these param-

TABLE 2. Regressions of length at first sea winter (L_{SW1}) and marine growth increment (ΔMG1) on smolt size for the predominant age-classes. Coefficients of determination have been adjusted for the degrees of freedom, and P is the probability that the slope (b) is different from zero.

	Age 1.1							Age 1.2						
	L_{SW1}				ΔMG1			L_{SW1}				ΔMG1		
	n	r^2 adjusted	b	P	r^2adjusted	b	P	n	r^2adjusted	b	P	r^2adjusted	b	P
Narcea														
1985	—	—	—	—	—	—	—	134	0.0	0.120	>0.5	0.13	−0.877	<0.0001
1986	125	0.11	0.490	<0.0001	0.12	−0.503	<0.0001	85	0.10	0.608	0.015	0.04	−0.395	0.0357
1987	76	0.14	0.517	0.0004	0.13	−0.487	0.0009	50	0.06	0.386	0.0534	0.16	−0.620	0.0026
1988	6	0.0	−0.352	0.59	0.45	−1.373	0.0872	75	0.13	0.702	0.0007	0.02	−0.296	0.14
1989	58	0.05	0.368	0.044	0.16	−0.630	0.0009	68	0.04	0.349	0.647	0.15	−0.656	0.0008
1990	51	0.0	0.046	0.72	0.54	−0.958	<0.0001	—	—	—	—	—	—	—
Esva														
1985	—	—	—	—	—	—	—	53	0.03	0.556	0.13	0.01	−0.448	0.22
1986	28	0.11	0.581	0.0463	0.05	−0.418	0.14	60	0.05	0.460	0.0496	0.07	−0.537	0.0238
1987	25	<0.01	0.284	0.32	0.18	−0.715	0.0185	51	<0.01	0.318	0.25	0.09	−0.680	0.017
1988	26	0.0	−0.066	0.85	0.24	−1.059	0.0071	49	0.22	0.877	0.0004	0.0	−0.124	0.59
1989	29	0.06	0.514	0.12	0.04	−0.490	0.13	52	0.0	0.044	0.9	0.11	−0.950	00092
1990	23	0.0	0.139	0.51	0.42	−0.868	0.0005	—	—	—	—	—	—	—
Cares														
1986	—	—	—	—	—	—	—	50	0.34	0.791	<0.0001	0.02	−0.212	0.18
1987	50	0.35	0.688	<0.0001	0.09	−0.312	0.0228	51	0.25	0.840	<0.0001	0.0	−0.166	0.41
1988	44	0.15	0.525	0.0059	0.12	−0.473	0.0123	50	0.18	0.614	0.0013	0.07	−0.387	0.0351
1989	37	0.11	0.547	0.0236	0.07	−0.451	0.056	52	0.25	0.752	<0.0001	0.02	−0.241	0.17
1990	47	0.31	0.759	<0.0001	0.03	−0.240	0.15	—	—	—	—	—	—	—

eters should not be altered in response to changes in the marine environment, which would only change the frequency of sea age-classes. The results presented here do not support such assumptions and suggest that neither size at the end of the first sea winter nor growth during the first year at sea determines age at maturity. Although these variables showed differences in relation to the final sea age, effects were not consistent among rivers. In addition, variations in ΔMG1 and L_{SW1} among years were clearly greater than the variation of these parameters between age-classes. For instance, length increments were much lower in 1989 and 1990 than in previous years regardless of sea age-class (Fig. 4 and 5A). Furthermore, the plot of ΔMG1 versus the 1SW/2SW ratio (rivers pooled) does not suggest any relationship between these variables (Fig. 5B). This lack of specific evidence for a marine growth influence on sea age at maturity is also consistent with the findings reported by Dempson et al. (1986). An alternative explanation is that the observed differences in marine growth could indirectly result from either genetic or ecological differences between sea age-groups. Synchrony in interannual variation in ΔMG1 among rivers suggests that sea growth is strongly influenced by some widespread environmental factor or that fish from neighboring stocks move through common feeding areas during the marine phase.

Large smolts from the Rivers Esva and Cares tended to mature earlier than small smolts of the same age. Similar results have been reported for steelhead (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*) (Ward and Slaney 1988), coho salmon (*Oncorhynchus kisutch*) (Bilton et al. 1982), sockeye salmon (*Oncorhynchus nerka*) (Hyatt and Stockner 1985), and Atlantic salmon, although genetic factors may be more important for Atlantic salmon (Nævdal 1983; Ritter et al. 1986; Chadwick et al. 1987; Skilbrei 1989). In the rivers sampled, most grilse are male whereas the majority of 2SW salmon are female (Prévost et al. 1991). Presmolt growth rates have been reported to be higher for male than for female cultured Atlantic salmon (Nævdal et al. 1983); therefore, this result may be caused by sex-specific growth rates. However, Chadwick et al. (1987) inferred from ovarian development a direct correlation between smolt length and early maturation. Sex-specific growth rates could not explain their result, as their analysis was restricted to females only. In addition, the size of the oocytes of nonmaturing presmolt females has been found to increase under favorable growth conditions during late winter (Adams and Thorpe 1989), and smolt size appears to be strongly correlated with size at the end of the first river winter in age-1 salmon (Nicieza and Braña 1993). Thus, "good" growing conditions resulting in larger smolts may also be responsible for the predisposition to mature earlier (Thorpe 1986, 1987) and account for the association between large size at smolting and maturation as grilse.

In salmonid fishes, it could be assumed that juvenile mortality increases as growth rates decrease, through, for example, the size dependence of predation risk (Werner et al. 1983). In this case, life-history theory predicts that high initial growth rates lead to early maturation (Roff 1984; Stearns and Crandall 1984; Stearns and Koella 1986). This pattern has been verified in nonanadromous salmonid populations (Jonsson 1977; Jonsson et al. 1984) and in many other fishes (Alm 1959; Helfman et al. 1987). In anadromous species, it becomes more complex to test life-history predictions because a variable fraction of the population may mature without going to sea (e.g., Ricker 1938; Dalley et al. 1983; Taylor 1989) whereas others delay maturation above the minimum physiological size. At the within-population level, the freshwater maturation of Atlantic salmon is associated with relatively high growth rates (Rowe and Thorpe 1990; Prévost et al. 1992), and thus the theory is verified. However, the fast growers also delay maturation, although they become the youngest smolts and therefore tend to reduce the total age at maturity. This apparent conflict may arise from the existence of sex-specific strategies. Presumably, males and females have evolved under different selection pressures, and therefore, different life-history solutions would be expected.

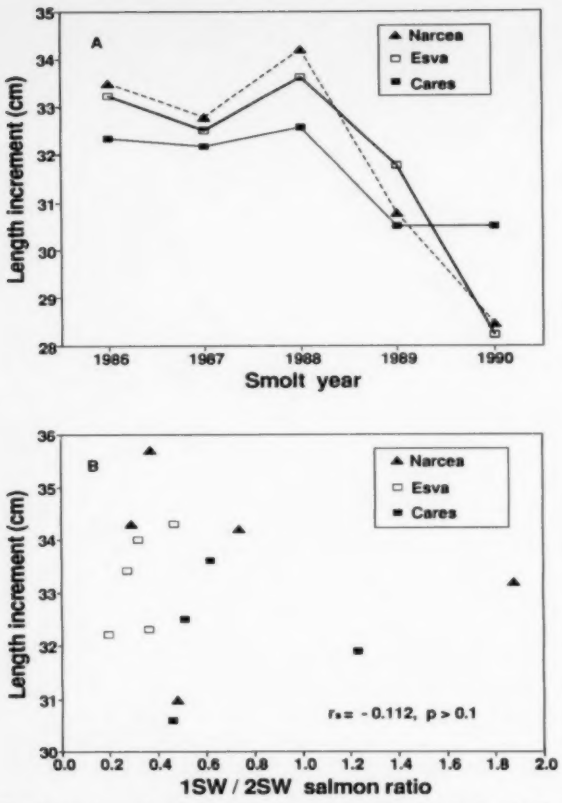


FIG. 5. (A) Annual variation in marine growth (ΔMG1) during the first sea year for salmon (ages 1.1 and 1.2 pooled) from the Rivers Narcea, Esva, and Cares and (B) relationship between the annual length increment and the $1SW_t/2SW_{t+1}$ salmon ratio in the angling fishery for the years considered for back-calculation.

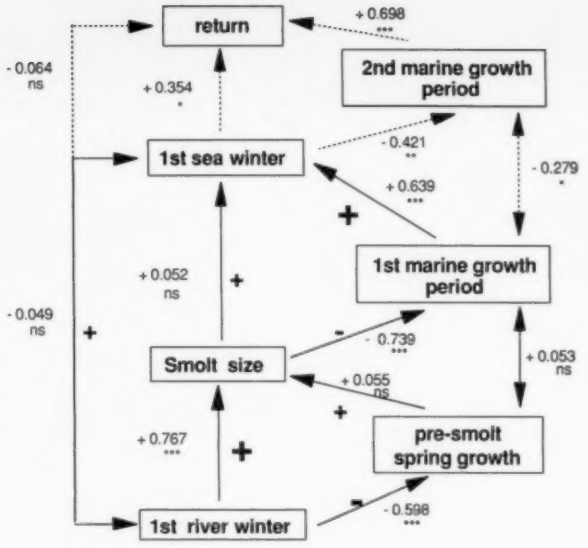


FIG. 6. Schematic model of growth relationships for Atlantic salmon based on the results reported here and in a previous study (Nicieza and Braña 1992). Single- and double-headed arrows indicate direct and indirect relationships, respectively. Symbols (+, –) show the overall sign of significant correlations (Pearson) observed consistently for different years, age-groups, and rivers. Large symbols correspond to pairs characterized by strong correlations whereas small symbols indicate weak but significant correlations in most of the subsets considered. Numeric values are the coefficients of correlation obtained for age 1.1 salmon from the River Narcea (smolt year 1990); $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; ns, not significant). Dotted arrows indicate relationships assayed exclusively for that combination.

Thus, whereas males generally mature in fresh water, females seldom do (Prouzet 1981; Hansen et al. 1989). Although life-history theory and optimization models may be useful to explain a part of the variability in age and size at maturity, the consideration of frequency-dependent selection is perhaps unavoidable in order to understand variability over the whole life cycle (Gross 1985, 1991; Holtby and Healey 1990). Optimization and frequency-dependent selection may act at different levels in the evolution of life cycles, regardless of the fact that the corresponding models are based on opposite assumptions.

The influence of smolt size appears to be stronger when parentage is a mix of grilse and MSW salmon than when releases originate only from one of these groups (Ritter et al. 1986). Sea age and sex structure of the three stocks studied here are similar, presumably favoring interbreeding between grilse (mostly male) and MSW salmon (mostly female). Therefore, it is unlikely that the different pattern observed in the River Narcea can be explained by parental age.

It has been suggested that smolt size has a positive influence on growth rates during the early marine phase (Ritter 1975; Lundqvist et al. 1988). In this study, however, smolt size was positively correlated with size at the first sea winter but was inversely correlated with the growth increment during the first year at sea. In most cases, these relationships were still significant within smolt years. The negative correlation between smolt length and ΔMG1 may be a direct consequence of smolts migrating later being larger than those migrating earlier, with the result that first marine growth period of the largest smolts would be shorter. However, this is not the only cause, as negative correlations were also found between ΔMG1 and length at the first river winter and for fish without freshwater spring growth (1^0 smolts) that presumably started their marine growth early and simultaneously. Skilbrei (1989) found that size after 1 yr in the sea was positively correlated with smolt size. He also documented an inverse correlation between smolt size and specific growth rates during the first 6 mo in seawater (Skilbrei 1990). Although growth compensation was greater for previously mature male parr, this effect was also noted for immature individuals, even though growth rates were adjusted for smolt size. In addition to these studies, Nævdal et al. (1979) noted growth compensation during the second marine growth period in Atlantic salmon. Compensatory growth has also been reported for Atlantic salmon presmolts (Nicieza and Braña 1993). In that case, growth compensation might be mainly accomplished by means of individual variation in the timing of migration. Skilbrei's (1990) study, in which time was fixed, showed that growth compensation also operates through shifts in growth rates. To our knowledge, the physiological basis of this "pure" compensatory growth is poorly understood (Ricker 1975) but may be related to higher efficiency and feeding motivation of the more starved fish (Dill and Fraser 1984; Dobson and Holmes 1984; Magnhagen 1988; Gotceitas and Godin

1991). The growth compensation found here is probably the result of two components: (1) variation in time of sea entry and (2) higher growth rates of the initially smaller fish (A.G. Nicieza and F. Braña, unpublished data). It supports the growth pattern described by Skilbrei (1990) for hatchery-reared stocks. Compensation appears to occur at each stage in the life cycle of Atlantic salmon (Fig. 6). This may be related to the fact that size is an important factor in determining sea survival of postsmolts (e.g., Bilton et al. 1982; Mathews and Ishida 1989; Henderson and Cass 1991) and at adult stages has multiple implications for fitness (Keenleyside and Dupuis 1988; van den Berghe and Gross 1989; Gross 1991; Hutchings 1991).

In conclusion, the positive relationship between smolt size and maturation as grilse and the lack of consistency among populations with regard to the differences observed in marine growth support previous suggestions that age at maturity may be determined prior to sea entry (Randall et al. 1986; Chadwick et al. 1987; Gross 1991). This fact may be viewed as a particular outcome of the general prediction that initial growth rates influence age at maturity. The differences observed in sea growth of wild salmon are presumably the consequence of grilse and MSW salmon having different migration routes (see Dempson et al. 1986). Our analysis does not discern "early" marine growth (e.g., length increment corresponding to the first five to eight marine circuli) but the entire first sea-growth period and is therefore unsuitable to evaluate whether growth rate during a short postsmolt period may influence age at maturity (Scarnecchia et al. 1989). In addition, our results provide evidence for a weak (but consistent over years and age-classes) negative relationship between smolt size and the subsequent growth up to the first sea winter, regardless of when maturation occurs.

Acknowledgments

We thank A.M. Nistal and J. Morales from the Servicio de Proceso de Imágenes of the University of Oviedo for valuable technical assistance and F.J. Pérez-Barbería and V. Vauclin who mounted some of the scale samples used in this study. We thank the staff of the Agencia del Medio Ambiente del Principado de Asturias and especially M. Pino Fernández-Rueda for allowing us to use the primary material for this study. The suggestions of E. Prévost and two anonymous reviewers contributed greatly to the improvement of the manuscript. We are also grateful to Sally McNeill for reviewing the English. This research was supported by project grants from FICYT and ICONA.

References

ADAMS, C.E., AND J.E. THORPE. 1989. Photoperiod and temperature effects on early development and reproductive investment in Atlantic salmon (*Salmo salar* L.). Aquaculture 79: 403–409.

ALLEN, K.R., R.L. SAUNDERS, AND P.F. ELSON. 1972. Marine growth of Atlantic salmon (*Salmo salar*) in the northwest Atlantic. J. Fish. Res. Board Can. 29: 1373–1380.

ALM, G. 1959. Connection between maturity, size, and age in fishes. Inst. Freshwater Res. Drottningholm Rep. 40: 5–145.

BILTON, H.T., D.F. ALDERDICE, AND J.T. SCHNUTE. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. Can. J. Fish. Aquat. Sci. 39: 426–442.

CHADWICK, E.M.P., R.R. CLAYTOR, C.E. LÉGER, AND R.L. SAUNDERS. 1987. Inverse correlation between ovarian development of Atlantic salmon (*Salmo salar*) smolts and sea age. Can. J. Fish. Aquat. Sci. 44: 1320–1325.

CHADWICK, E.M.P., R.G. RANDALL, AND C. LÉGER. 1986. Ovarian development of Atlantic salmon (*Salmo salar*) smolts and age at maturity, p. 15–23. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

DALLEY, E.L., C.W. ANDREWS, AND J.M. GREEN. 1983. Precocious male Atlantic salmon parr (*Salmo salar*) in insular Newfoundland. Can. J. Fish. Aquat. Sci. 40: 647–652.

DEMPSON, J.B., R.A. MYERS, AND D.G. REDDIN. 1986. Age at first maturity of Atlantic salmon (*Salmo salar*) – influences of the marine environment, p. 79–89. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

DILL, L.M., AND D.F. FRASER. 1984. Risk of predation and the feeding behavior of juvenile coho salmon (*Oncorhynchus kisutch*). Behav. Ecol. Sociobiol. 16: 65–71.

DOBSON, S.H., AND R.M. HOLMES. 1984. Compensatory growth in the rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 25: 649–656.

FRANCIS, R.I.I.C. 1990. Back-calculation of fish length: a critical review. J. Fish Biol. 36: 883–902.

GARDNER, M.L. 1976. A review of factors which may influence the sea-age and maturation of Atlantic salmon *Salmo salar*. J. Fish Biol. 9: 289–327.

GJERDE, B. 1984. Response to individual selection for age at sexual maturity in Atlantic salmon. Aquaculture 38: 229–240.

GJERDE, B., AND T. GJEDREM. 1984. Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. Aquaculture 36: 97–110.

GLEBE, B.D., AND R.L. SAUNDERS. 1986. Genetic factors in sexual maturity of cultured Atlantic salmon (*Salmo salar*) parr and adults reared in sea cages, p. 24–29. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

GOTCEITAS, V., AND J.G. GODIN. 1991. Foraging under the risk of predation in juvenile Atlantic salmon (*Salmo salar* L.): effects of social status and hunger. Behav. Ecol. Sociobiol. 29: 255–261.

GROSS, M.R. 1985. Disruptive selection for alternative life histories in salmon. Nature (Lond.) 313: 47–48.

GROSS, M.R. 1991. Salmon breeding behavior and life history evolution in changing environments. Ecology 72: 1880–1886.

HANSEN, L.P., B. JONSSON, R.I.G. MORGAN, AND J.E. THORPE. 1989. Influence of parr maturity on emigration of smolting Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 46: 410–415.

HELFMAN, G.S., D.E. FACEY, L. STANTON-HALES, AND E.L. BOZEMAN. 1987. Reproductive ecology of the American eel. Am. Fish. Soc. Symp. 1: 42–56.

HENDERSON, M.A., AND A.J. CASS. 1991. Effect of smolt size on smolt-to-adult survival for Chilko Lake sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. 48: 988–994.

HEULIN, B. 1985. Maturité sexuelle et âge la première reproduction dans une population de plaine de *Lacerta vivipara*. Can. J. Zool. 63: 1773–1777.

HOLTBY, L.B., B.C. ANDERSEN, AND R.K. KADOWAKI. 1990. Importance of smolt size and early ocean growth to interannual variability in marine survival of coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 47: 2181–2194.

HOLTBY, L.B., AND M.C. HEALEY. 1990. Sex-specific life history tactics and risk-taking in coho salmon. Ecology 71: 678–690.

HUTCHINGS, J.A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. Evolution 45: 1162–1168.

HYATT, K.D., AND J.G. STOCKNER. 1985. Responses of sockeye salmon (*Oncorhynchus nerka*) to fertilization of British Columbia coastal lakes. Can. J. Fish. Aquat. Sci. 42: 320–331.

JONSSON, B. 1977. Demographic strategy in a brown trout population in Western Norway. Zool. Scr. 6: 255–263.

JONSSON, B., K. HINDAR, AND T.G. NORTHCOTE. 1984. Optimal age at sexual maturity of sympatric and experimentally allopatric cutthroat trout and Dolly Varden charr. Oecologia (Berl.) 61: 319–325.

KEENLEYSIDE, M.H.A., AND M.C. DUPUIS. 1988. Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*). Can. J. Zool. 66: 262–265.

LUNDQVIST, H., W.C. CLARKE, AND H. JOHANSSON. 1988. The influence of sexual maturation on survival to adulthood of river stocked Baltic salmon, *Salmo salar*, smolts. Holarct. Ecol. 11: 60–69.

MAGNHAGEN, C. 1988. Predation risk and foraging in juvenile pink (*Oncochrhynchus gorbuscha*) and chum salmon (*O. keta*). Can. J. Fish. Aquat. Sci. 45: 592–596.

MATHEWS, S.B., AND Y. ISHIDA. 1989. Survival, ocean growth, and ocean distribution of differentially timed releases of hatchery coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 46: 1216–1226.

NÆVDAL, G. 1983. Genetic factors in connection with age at maturity. Aquaculture 33: 97–106.

NÆVDAL, G., O. BJERK, M. HOLM, R. LEROY, AND D. MOLLER. 1979. Growth rate and age at sexual maturity of Atlantic salmon smoltifying aged one and two years. FiskDir. Skr. Ser. HavUnders. 17: 11–17.

NÆVDAL, G., M. HOLM, O. INGEBRIGTSEN, AND D. MOLLER. 1978. Variation in age at first spawning in Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 35: 145–147.

NÆVDAL G., R. LEROY, AND D. MOLLER. 1983. Sources of variation in weight and length of Atlantic salmon. FiskDir. Skr. Ser. HavUnders. 17: 359–366.

NICIEZA, A.G., AND F. BRAÑA. 1993. Compensatory growth and optimum size in one-year-old smolts of Atlantic salmon (*Salmo salar*), p. 225–237. *In* R.J. Gibson and R.E. Cutting [ed.] Production of juvenile Atlantic salmon, *Salmo salar*, in natural waters. Can. Spec. Publ. Fish. Aquat. Sci. 118.

NICIEZA, A.G., F. BRAÑA, AND M.M. TOLEDO. 1991. Development of length-bimodality and smolting in wild stocks of Atlantic salmon, *Salmo salar* L., under different growth conditions. J. Fish Biol. 38: 509–523.

NICIEZA, A.G., M.M. TOLEDO, AND F. BRAÑA. 1990. Capturas de Salmón Atlántico en los ríos asturianos en el período 1953–1989. Variaciones de abundancia y estructura de edades de mar. Biobas. Rev. Biol. Univ. Oviedo. IV: 91 p.

PETERMAN, R.M. 1982. Nonlinear relation between smolts and adults in Babine Lake sockeye salmon (*Oncorhynchus nerka*) and implications for other salmonid populations. Can. J. Fish. Aquat. Sci. 39: 904–913.

PETERMAN, R.M. 1985. Patterns of interannual variation in age at maturity of sockeye salmon (*Oncorhynchus nerka*) in Alaska and British Columbia. Can. J. Fish. Aquat. Sci. 42: 1595–1607.

PORTER, T.R., M.C. HEALEY, AND M.F. O'CONNELL (WITH E.T. BAUM, A.T. BIELAK, AND Y. CÔTÉ). 1986. Implications of varying the sea age at maturity of Atlantic salmon (*Salmo salar*) on yield to the fisheries, p. 110–117. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

PRÉVOST, E., E.M.P. CHADWICK, AND R.R. CLAYTOR. 1992. Influence of size, winter duration and density on sexual maturation of Atlantic salmon (*Salmo salar*) juveniles in Little Codroy River (southwest Newfoundland). J. Fish Biol. 41: 1013–1019.

PRÉVOST, E., V. VAUCLIN, J.L. BAGLINIÈRE, F. BRAÑA-VIGIL, AND A.G. NICIEZA. 1991. Application d'une méthode de détermination externe du sexe chez le Saumon Atlantique (*Salmo salar* L.) dans les rivières des Asturies (Espagne). Bull. Fr. Pêche Piscic. 323: 149–159.

PROUZET, P. 1981. Observation d'une femelle de tacon de saumon atlantique (*Salmo salar* L.) perrenue à maturité sexuelle en rivière. Bull. Fr. Pêche Piscic. 282: 16–19.

RANDALL, R.G., J.E. THORPE, R.J. GIBSON, AND D.G. REDDIN. 1986. Biological factors affecting age at maturity in Atlantic salmon (*Salmo salar*), p. 90–96. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

RICKER, W.E. 1938. "Residual" and kokanee salmon in Cultus Lake. J. Fish. Res. Board Can. 4: 192–218.

RICKER, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 382 p.

RITTER, J.A. 1975. Relationships of smolt size and age with age at first maturity in Atlantic salmon. Res. Dev. Br. Environ. Can. Tech. Rep. Ser. No. MAR/F-75-5: 7 p.

RITTER, J.A, G.J. FARMER, R.K. MISRA, T.R. GOFF, J.K. BAILEY, AND E.T. BAUM. 1986. Parental influences and smolt size and sex ratio effects on sea age at first maturity of Atlantic salmon (*Salmo salar*), p. 30–38. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

ROFF, D.A. 1984. The evolution of life history parameters in teleosts. Can. J. Fish. Aquat. Sci. 41: 989–1000.

ROWE, D.K., AND J.E. THORPE. 1990. Suppression of maturation in male Atlantic salmon (*Salmo salar* L.) parr by reduction in feeding and growth during spring months. Aquaculture 86: 291–313.

SAUNDERS, R.L., E.B. HENDERSON, B. GLEBE, AND E.J. LOUDENSLAGER. 1983. Evidence of a major environmental component in determination of the grilse:larger salmon ratio in Atlantic salmon (*Salmo salar*). Aquaculture 33: 107–118.

SCARNECCHIA, D.L., A. ÍSAKSSON, AND S.A. WHITE. 1989. Effects of oceanic variations and the West Greenland fishery on age at maturity of Icelandic west coast stocks of Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 46: 16–27.

SCHAFFER, W.M. 1974. Selection for optimal life histories: the effects of age structure. Ecology 55: 291–303.

SCHAFFER, W.M., AND P.F. ELSON. 1975. The adaptive significance of variations in life history among local populations of Atlantic salmon in North America. Ecology 56: 577–590.

SEMLITSCH, R.D., D.E. SCOTT, AND J.H.K. PECHMANN. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology 69: 184–192.

SKILBREI, O.T. 1989. Relationships between smolt length and growth and maturation in the sea of individually tagged Atlantic salmon (*Salmo salar*). Aquaculture 83: 95–108.

SKILBREI, O.T. 1990. Compensatory sea growth of male Atlantic salmon, *Salmo salar* L., which previously matured as parr. J. Fish Biol. 37: 425–435.

SMITH, D.C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68: 344–350.

STEARNS, S.C., AND R.E. CRANDALL. 1984. Plasticity for age and size at maturity: a life-history response to unavoidable stress, p. 13–33. *In* G.W. Potts and R.J. Wootton [ed.] Fish reproduction: strategies and tactics. Academic Press, London. 410 p.

STEARNS, S.C., AND J.C. KOELLA. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40: 893–913.

TAYLOR, E.B. 1989. Precocial male maturation in laboratory-reared populations of chinook salmon, *Oncorhynchus tshawytscha*. Can. J. Zool. 67: 1665–1669.

THORPE, J.E. 1986. Age at first maturity in Atlantic salmon, *Salmo salar*: freshwater period influences and conflicts with smolting, p. 7–14. *In* D.J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

THORPE, J.E. 1987. Smolting versus residency: developmental conflict in salmonids. Am. Fish. Soc. Symp. 1: 244–252.

VAN DEN BERGHE, E.P., AND M.R. GROSS. 1989. Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution 43: 125–140.

WARD, B.R., AND P.A. SLANEY. 1988. Life history and smolt-to-adult survival of Keogh River steelhead trout (*Salmo gairdneri*) and the relationship to smolt size. Can. J. Fish. Aquat. Sci. 45: 1110–1122.

WERNER, E.E., J.F. GILLIAM, D.J. HALL, AND G.G. MITTELBACH. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology 64: 1540–1548.

Heterozygosity and Morphological Variability in the Orange Roughy, *Hoplostethus atlanticus* (Teleostei: Trachichthyidae)

Robert D. Ward and Nicholas G. Elliott

CSIRO Division of Fisheries, GPO Box 1538, Hobart, Tasmania 7001, Australia

Ward, R.D., and N.G. Elliott. 1993. Heterozygosity and morphological variability in the orange roughy, *Hoplostethus atlanticus* (Teleostei: Trachichthyidae). Can. J. Fish. Aquat. Sci. 50: 1641–1649.

Eleven polymorphic loci were screened in six southern Australian populations of the deepwater teleost *Hoplostethus atlanticus*, the orange roughy. Associations between heterozygosity and morphological variability were examined. No significant correlations between heterozygosity at individual loci and length were observed. Homozygotes at *ADA** had significantly greater length variance than heterozygotes in one population whereas in another population, homozygotes for *PGM-2** had significantly reduced length variance compared with heterozygotes. No other single locus/length variance comparisons were significant. One of the six populations showed a significant positive regression of individual length on multilocus heterozygosity; regressions in the other five populations were nonsignificant and variable in slope direction. No significant correlations were detected between multilocus heterozygosity category and mean length or coefficient of variance of length. Three of the populations were also examined for variation in four meristic characters and one measure of bilateral asymmetry: no consistent associations were detected between individual or multilocus heterozygosity and mean or variance of these characters. The data provide little evidence for significant relationships between individual or multilocus heterozygosity and morphological variability in orange roughy.

Dans six populations du sud de l'Australie du poisson téléostéen *Hoplostethus atlanticus*, l'hoplostète orange, on a examiné 11 locus polymorphes. L'association entre l'hétérozygotie et la variabilité morphologique a été étudiée. Aucune corrélation entre l'hétérozygotie de chaque locus et la longueur n'a été observée. Dans une population, les homozygotes *ADA** variaient considérablement plus en longueur que les hétérozygotes, tandis que dans une autre population, les homozygotes *PGM** variaient moins en longueur que les hétérozygotes. Aucune autre comparaison entre la variabilité de la longueur et un locus n'était significative. Dans l'une des six populations, on a observé une régression significativement positive entre la longueur individuelle et l'hétérozygotie en plusieurs locus; dans les cinq autres populations, les régressions n'étaient pas significatives, et la direction de la pente était variable. Aucune corrélation significative n'a été décelée entre l'hétérozygotie multiple et la longueur moyenne ou le coefficient de variabilité de la longueur. En outre, dans trois des populations, on a aussi examiné la variation de quatre caractères méristiques et une mesure d'asymétrie bilatérale : aucune association constante n'a été décelée entre l'hétérozygotie simple ou l'hétérozygotie multiple et la moyenne ou la variance de ces caractères. Les données dévoilent peu de rapports significatifs entre l'hétérozygotie simple ou multiple et la variabilité morphologique chez l'hoplostète orange.

Received August 17, 1992
Accepted February 16, 1993
(JB602)

Reçu le 17 août 1992
Accepté le 16 février 1993

Over the last 10 yr, the relationships between morphological and genetic variability have been explored in an ever increasing number of plant and animal species (earlier work summarised in Mitton and Grant 1984). In these studies, small numbers of polymorphic loci (typically less than 10) have been examined for correlations between individual multilocus heterozygosity and traits such as size, growth rate, meristic variation, and asymmetry. Associations have been detected in many, but not all, species investigated. Because heterozygosity at such a small number of polymorphic loci has a very small correlation with overall genomic heterozygosity in outbreeding species (Mitton and Pierce 1980; Chakraborty 1981), explanations based on genomic genetic homeostasis (using the concepts of Lerner 1954) seem untenable, and either the loci themselves or linked loci must be the targets of selection (see Chakraborty 1987).

Diverse associations have been detected within natural populations of sexually reproducing fish. Decreased morphological variance with increasing heterozygosity has been found in mummichog, *Fundulus heteroclitus* (Mitton 1978), but not in plaice, *Pleuronectes platessa* (McAndrew et al. 1982), high cockscomb, *Anoplarchus purpurescens* (Yoshiyama and Sassaman 1983), or chum, *Oncorhynchus keta*, and pink salmon, *Oncorhynchus gorbuscha* (Beacham and Withler 1985a, 1985b). In the Atlantic herring, *Clupea harengus*, decreasing morphological variance with increasing heterozygosity was found by King (1985) but not by Ryman et al. (1984). Correlations between size and heterozygosity were found in Atlantic herring (King 1985) but not in plaice (McAndrew et al. 1986) or pink salmon (Beacham and Withler 1985b). In rainbow trout, *Oncorhynchus mykiss*, the relationship between size and multilocus heterozygosity appears to change with age (Ferguson 1992, and references therein).

The degree of asymmetry of bilateral characters has also been used as a measure of developmental homeostasis. For example, a significant reduction in asymmetry with increasing individual heterozygosity has been recorded in natural populations of cut-

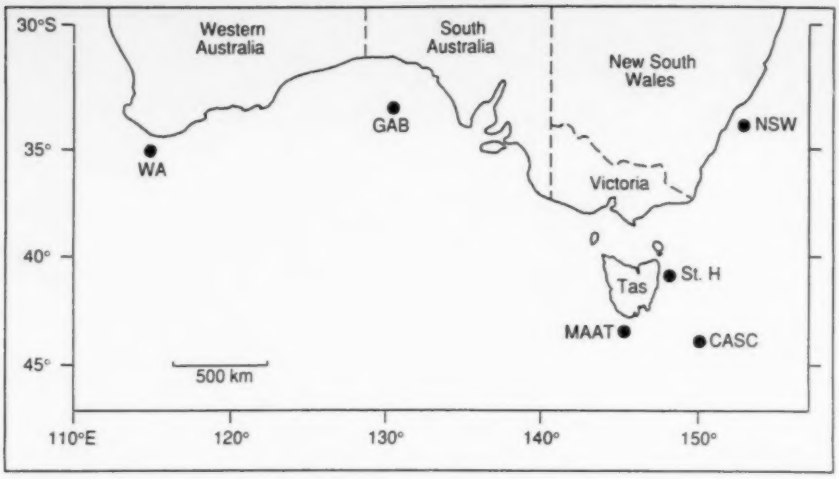


FIG. 1. Location of sampling sites. WA, West Australia; GAB, Great Australian Bight; MAAT, Maatsuyker; CASC, Cascade Plateau; St. H, St. Helens; NSW, New South Wales.

throat trout, *Oncorhynchus clarki*, while nonsignificant (but still negative) correlations were observed in wild rainbow trout (some domesticated strains showed significant negative correlations) and brook trout, *Salvelinus fontinalis* (Leary et al. 1984). No associations between asymmetry and heterozygosity were recorded for chum and pink salmon (Beacham and Withler 1987).

It thus appears that in some fish species, heterozygosity is positively correlated with size and negatively correlated with morphological variance but that in other species, no such relationships are found. In order to ascertain whether such correlations are widespread, it is necessary to extend the number of species assayed. Here, we examine southern Australian populations of the orange roughy, *Hoplostethus atlanticus*, for relationships between single and multilocus heterozygosity and length, four meristic characters, and one measure of asymmetry. This is a deep-sea species (approximately 800–1200 m) with high average allozyme heterozygosity (approximately 13%, Elliott and Ward 1992) and is the subject of an important fishery in Australian and New Zealand waters.

Materials and Methods

Samples collected in 1990–91 from six areas (Fig. 1) off the southern Australian coast were analysed for variation at 11 polymorphic allozyme loci (*LDH-1**, *MDH-1**, *IDH-1**, *CK**, *EST-1**, *ADA**, *MPI**, *GPI-1**, *GPI-2**, *PGM-1**, *PGM-2**). With the exception of the St. Helens (St. H) sample, all fish were obtained from commercial catches with whole frozen or iced fish sent to the laboratory. The St. H fish were collected by RV *Southern Surveyor* and were sampled at sea. Details of biochemical methods used, along with allele frequencies and an analysis of the extent of genetic differentiation among sites, are given in Elliott and Ward (1992). Gene nomenclature follows the recommendations of Shaklee et al. (1990).

The standard length (to an accuracy of 1 mm except for the St. Helens fish which were to an accuracy of 5 mm) and sex of the great majority of fish collected were recorded. In addition, for fish from three of the areas (Cascade Plateau (CASC), Great Australian Bight (GAB), Maatsuyker (MAAT)), the following meristic counts were made: dorsal fin spines (DS), dorsal fin rays (DR), anal fin spines (AS), anal fin rays (AR), left pectoral fin rays, unbranched (dorsal) (LPI), left pectoral fin rays, branched (LPII), left pectoral fin rays, unbranched (ventral) (LPIII), right pectoral fin rays, unbranched (dorsal) (RPI), right pectoral fin rays, branched (RPII), and right pectoral fin rays, unbranched (ventral) (RPIII). Asymmetry (ASY) was studied by comparing the total number of left and right pectoral fin rays (LP and RP where LP = LPI + LPII + LPIII, RP = RPI + RPII + RPIII, and ASY = |LP − RP|).

Statistical analysis was carried out using SYSTAT 5.1. The relationships between individual locus heterozygosity or homozygosity and the means and variances of all characters except ASY used *t*-tests (using separate variances where the variances were unequal and pooled variances where the variances were equal) and variance ratio (*F*) tests, respectively. The *F*-tests were two-tailed, testing the hypotheses $H_0 : \sigma^2$ homozygotes = σ^2 heterozygotes, with $H_1 : \sigma^2$ homozygotes ≠ σ^2 heterozygotes. Mann–Whitney (nonparametric) tests were also carried out and were in close agreement with the *t*-test results: generally, only the latter are presented here. In contrast with the other quantitative characters, the parameter ASY had a Poisson distribution, with means very similar to variances. The relationship between the means of this variable and individual locus homozygosity or heterozygosity was studied using a Mann–Whitney test.

Each fish was also allotted a multilocus heterozygosity score (*H*) equal to the number of heterozygous loci in that fish. In principle, this could range from 0 to 11; in practice, no fish with $H > 9$ was detected. Relationships between *H* and most morphological variables were generally examined through ANOVAs and Pearson correlation coefficients, although on occasion (and routinely for ASY) the nonparametric Spearman correlation was estimated.

For many of the analyses, a large number of simultaneous comparisons (involving, for example, different loci and different sites) was performed. In order to adjust significance levels for

TABLE 1. Mean lengths (cm, standard length) of fish from the six different sites.

Site	Males			Female			Total		
	n	Mean length	SD	*n*	Mean length	SD	*n*[a]	Mean length	SD
CASC	50	40.46	5.91	51	40.55	9.64	101	40.51	7.98
GAB	39	33.90	3.83	44	34.46	5.29	85	34.21	4.63
St. H	31	35.29	2.31	83	36.64	3.10	151	35.83	3.04
MAAT	54	33.11	4.18	56	34.29	4.72	110	33.71	4.48
NSW	57	30.84	2.64	112	32.74	3.32	169	32.10	3.23
WA	50	40.44	3.55	49	42.02	4.82	99	41.22	4.28
Overall	283	35.61	5.49	395	36.13	6.07	715	35.84	5.73

Tukey's honest significance difference (HSD) analysis on total numbers; pairs of populations that are not significantly different in mean size at $P < 0.05$ are linked

← Increasing size

WA CASC St. H GAB MAAT NSW

[a] Includes some unsexed fish.

TABLE 2. Summary statistics of the meristic traits and the two measures of asymmetry.

Trait	CASC			GAB			MAAT			Pooled samples		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
DS	128	4.781	0.614	85	5.024	0.487	110	4.482	0.655	323	4.845	0.606
DR	128	18.211	0.706	84	17.726	0.665	110	17.755	0.719	322	17.929	0.735
AS	129	2.884	0.345	85	2.871	0.338	109	2.917	0.277	323	2.892	0.321
AR	129	11.217	0.661	85	10.965	0.392	109	11.028	0.372	323	11.087	0.522
LP	128	19.063	0.750	85	19.094	0.648	110	18.891	0.640	323	19.012	0.691
LPI	129	1.984	0.124	85	2.000	0.000	110	1.991	0.095	324	1.991	0.096
LPII	128	15.414	0.737	85	15.212	0.656	110	15.236	0.690	323	15.300	0.705
LPIII	129	1.659	0.476	85	1.882	0.359	110	1.664	0.494	324	1.719	0.464
RP	131	19.038	0.514	84	19.179	0.679	110	18.809	0.449	325	18.997	0.705
RPI	131	1.992	0.087	84	2.000	0.000	110	1.982	0.191	325	1.991	0.124
RPII	131	15.397	0.664	84	15.357	0.688	110	15.191	0.628	325	15.317	0.663
RPIII	131	1.649	0.495	84	1.821	0.415	110	1.636	0.483	325	1.689	0.477
LP-RP	128	0.031	0.731	84	–0.083	0.496	110	0.082	0.706	322	0.019	0.670
ASY	128	0.375	0.627	84	0.250	0.436	110	0.355	0.615	322	0.335	0.579

multiple tests, the standard Bonferroni technique was used (Miller 1980). The predetermined significance level, α (where $\alpha = 0.05$), was divided by the number of tests to obtain a corrected significance level, α'. A result was considered significant at the 0.05 level if the test result had a probability less than α'.

Results

Genetic Variation

The extent of genetic differentiation among fish from the six different areas is extremely small, with the coefficient of genetic differentiation, G_{ST} (Nei 1973), over the 11 polymorphic loci estimated at no greater than 0.001 (Elliott and Ward 1992). Populations were in Hardy–Weinberg equilibrium. A two-way ANOVA showed that *H* was not significantly associated with site ($P = 0.600$) or with sex ($P = 0.478$), nor was there a site by sex interaction ($P = 0.630$).

Morphology

A two-way ANOVA showed that there was significant variation in length (Table 1) between both sites ($P < 0.0001$) and sexes ($P = 0.0036$), but there was no site by sex interaction ($P = 0.725$). At all sites, females were on average slightly larger than males, with overall mean sizes of 36.13 and 35.61 cm, respectively. A Tukey HSD analysis (Table 1) showed that the mean lengths of fish from CASC and West Australia (WA) were not significantly different from one another but were greater than those of fish from the other sites. New South Wales (NSW) fish were on average the smallest and included more small and immature specimens than collections from the other sites.

There was evidence of bimodality of length (not related to sex) at some sites, although this was not evident in the pooled data, which had a normal distribution. However, nonparametric analysis (Kruskal–Wallis) still showed significant variation in length between sites ($P < 0.001$) and between sexes ($P = 0.0042$).

Two characters, LPI and RPI, showed insufficient variation (Table 2) for statistical analysis and are not considered further. In addition, some of the remaining characters were significantly correlated with one another. Those pairs that were significantly ($P < 0.05$) correlated (following a Pearson correlation analysis using Bonferroni probabilities) in more than one of the three population samples tested were DS and DR, LPII and RPII, and LPIII and RPIII. These pairs of characters cannot be considered as independent. There is also evidence that the pairs of characters LPII and LPIII and RPII and RPIII are not independent of each other. Here the correlations are uniformly negative and, when data across populations are pooled, highly significant (RPII and RPIII: $n = 325$, $r = -0.273$, $P < 0.001$; LPII and LPIII: $n = 324$, $r = -0.343$, $P < 0.001$). This indicates compensation processes for total numbers of pectoral fin rays, such that if the number of ventrally located unbranched pectoral fin rays is decreased, the

TABLE 3. Relationships between individual locus heterozygosity–homozygosity and mean and variance of length at each site. *P*-values were $\alpha' = 0.0008$ and for the pooled data, $\alpha' = 0.0045$. ns* indicates $P < 0.05$ but greater than α'.

		CASC			GAB			St. H		
Locus	State	*n*	Mean	Variance	*n*	Mean	Variance	*n*	Mean	Variance
*GPI-1**	hom	34	41.59	55.04	28	32.86	20.58	37	36.78	4.84
	het	67	39.96	67.91	56	35.16	16.57	112	35.68	9.21
	P		ns	ns		ns*	ns		ns*	ns*
*GPI-2**	hom	97	40.51	63.98	78	34.17	22.30	139	35.86	9.78
	het	4	40.50	72.34	7	34.71	13.57	12	35.58	3.54
	P		ns	ns		ns	ns		ns	ns
*MDH-1**	hom	58	40.69	64.53	48	33.88	25.56	86	35.64	11.85
	het	43	40.26	63.71	37	34.65	16.29	65	36.09	5.84
	P		ns	ns		ns	ns		ns	ns*
*PGM-1**	hom	41	38.73	81.70	38	35.00	20.00	89	35.46	8.29
	het	60	41.72	48.72	47	33.57	22.12	62	36.37	10.30
	P		ns	ns		ns	ns		ns	ns
*PGM-2**	hom	81	41.09	56.69	68	34.27	22.92	113	36.21	8.10
	het	20	38.15	88.77	17	34.00	16.50	38	34.71	11.29
	P		ns	ns		ns	ns		ns*	ns
*CK**	hom	47	40.02	82.25	47	35.40	11.64	77	36.60	8.14
	het	54	40.93	48.22	37	33.11	25.60	72	35.26	7.69
	P		ns	ns		ns*	ns*		ns*	ns
*EST-1**	hom	41	39.02	86.47	38	34.18	26.64	74	35.95	7.70
	het	60	41.52	46.59	47	34.23	17.71	77	35.73	10.86
	P		ns	ns*		ns	ns	ns		ns
*LDH-1**	hom	71	40.65	64.88	60	34.05	21.60	125	35.74	9.75
	het	30	40.17	62.49	24	35.13	15.42	26	36.31	6.94
	P		ns	ns		ns	ns		ns	ns
*ADA**	hom	68	39.21	78.82	57	33.84	19.28	102	36.03	10.43
	het	33	43.18	22.72	28	34.96	25.82	49	35.43	6.75
	P		ns*	0.0002		ns	ns		ns	ns
*IDH-1**	hom	86	40.13	68.29	74	34.03	22.47	120	35.90	8.38
	het	15	42.67	33.66	11	35.46	14.07	31	35.58	12.98
	P		ns	ns		ns	ns		ns	ns
*MPI**	hom	81	40.33	63.30	68	34.21	24.02	132	35.64	9.73
	het	20	41.20	67.54	17	34.24	11.94	19	37.16	4.25
	P		ns	ns		ns	ns		ns*	ns*

number of branched fin rays increases. Consequently, the three fin ray counts for each pectoral fin were combined in later analyses, producing two new variables, LP (LP = LPI + LPII + LPIII) and RP (RP = RPI + RPII + RPIII). These two variables were, unsurprisingly given the earlier analysis, significantly correlated with each other.

Considerations of these tests of independence meant that in subsequent statistical tests, only the following meristics (the ASY analyses were carried out separately) were considered: DR, AS, AR, and LP. Two-way ANOVAs showed that of these characters, there were significant differences among sites for DR ($P < 0.001$) and AR ($P = 0.012$). The mean values of DR and AR were greater for the CASC sample than either the GAB or MAAT samples (Table 2). LP bordered on significance ($P = 0.062$). For none of these characters were there any sex differences or site by sex interactions. These conclusions also held following nonparametric (Kruskal–Wallis) analyses.

Mean counts of pectoral fin rays on the left and right body sides do not differ significantly. The parameter ASY shows that, on average, the two sides differ by about 0.3 ray (Table 2). For neither parameter is there a significant site or sex difference.

There were no significant correlations between length and any of the four meristic characters or ASY.

Genetics and Morphology

There were no associations between heterozygosity and sex, and the two sexes were pooled. However, there was some evidence of interactions between morphological and genetic variables at the different sites. For example, a preliminary analysis showed that there was a significant ($P = 0.025$) treatment (site) by covariate (multilocus heterozygosity, *H*) interaction with respect to the dependent variable length, meaning that the different sites had different slopes of length on *H* and making an analysis of covariance inappropriate. Most of the ensuing analyses consider collections from the different sites separately.

Heterozygosity and length

Means and variances of length for fish homozygous or heterozygous for each locus ($n = 11$) at each site ($n = 6$) were estimated (Table 3). A total of 66 *t*-tests analysed differences in mean length between homozygotes and heterozygotes for each locus/site combination, and after correcting α levels for the number of tests ($\alpha = 0.05$, $\alpha' = \alpha/66 = 0.0008$), not one of the tests was significant. Application of the Mann–Whitney *U*-test gave the same result. Overall, 26 cases showed that the mean length of homozygotes exceeded heterozygotes; 40 cases showed the reverse. This is not significantly different from a ratio

tested for significance against α' where $\alpha' = \alpha/n$, n being the number of simultaneous tests applied. For individual locus–population comparisons,

MAAT			NSW			WA			All sites pooled		
n	Mean	Variance	*n*	Mean	Variance	*n*	Mean	Variance	*n*	Mean	Variance
38	33.68	20.87	66	31.77	7.32	34	40.44	22.62	237	35.64	33.58
72	33.72	19.95	103	32.33	12.39	65	41.63	15.83	475	36.01	31.74
	ns	ns		ns	ns*		ns	ns		ns	ns
96	33.85	20.11	144	32.08	10.94	91	41.06	19.32	645	35.94	33.59
14	32.71	20.22	25	32.24	7.77	8	43.13	2.98	70	34.87	24.98
	ns	ns		ns	ns		ns*	ns*		ns	ns
64	33.45	21.81	104	31.83	9.13	60	40.55	16.25	420	35.56	33.54
46	34.07	17.88	65	32.54	12.35	39	42.26	20.09	295	36.23	31.64
	ns	ns		ns	ns		ns	ns		ns	ns
51	34.00	17.12	66	32.59	6.86	51	40.35	11.88	336	35.77	26.64
59	33.46	22.84	101	31.84	12.45	45	42.53	21.48	374	35.94	38.42
	ns	ns		ns	ns*		ns*	ns*		ns	ns
92	33.79	20.08	124	32.22	7.95	77	41.58	18.53	555	36.14	32.68
18	32.28	21.03	45	31.78	17.45	21	40.43	11.66	159	34.83	32.15
	ns	ns		ns	0.0008		ns	ns		ns*	ns
59	32.83	17.28	98	31.85	11.66	43	40.84	21.52	371	35.52	32.38
51	34.73	21.76	69	32.44	8.43	56	41.52	15.93	339	36.31	32.10
	ns*	ns		ns	ns		ns	ns		ns	ns
45	34.76	21.23	68	32.57	7.80	36	40.75	17.51	302	35.78	30.70
65	32.99	18.29	101	31.78	12.03	63	41.49	18.80	413	35.88	34.45
	ns*	ns		ns	ns		ns	ns		ns	ns
79	34.01	21.01	135	31.96	9.37	71	40.90	17.49	541	35.68	31.64
31	32.94	17.46	34	32.68	14.59	28	42.04	20.04	173	36.42	35.38
	ns	ns		ns	ns		ns	ns		ns	ns
76	33.58	20.17	116	32.10	12.08	67	41.09	13.21	486	35.60	33.02
34	34.00	20.37	53	32.09	6.97	32	41.50	29.55	229	36.35	32.15
	ns	ns		ns	ns*		ns	ns*		ns	ns
98	33.52	20.31	150	31.94	9.36	82	41.01	19.95	610	35.61	33.32
12	35.25	16.93	19	33.37	17.91	16	41.88	11.18	104	37.12	28.30
	ns	ns		ns	ns*		ns	ns		ns*	ns
92	34.01	18.67	136	31.93	10.73	68	40.82	18.98	577	35.61	31.65
18	32.17	25.80	33	32.79	8.86	31	42.10	16.16	138	36.80	36.83
	ns	ns		ns	ns		ns	ns		ns*	ns

of 1:1. The WA sample was unusual, as 10 of the 11 locus tests showed heterozygote mean length exceeding that of homozygotes. In the pooled data, none of the 11 locus tests had probabilities less than $\alpha'(\alpha' = 0.05/11 = 0.0045)$. However, for nine of these 11 loci, mean lengths of heterozygotes exceeded homozygotes, a ratio significantly different from the expected 1:1 ($P = 0.035$).

F-tests were used to compare single locus length variances. Only two tests had $P < \alpha'$. One was *PGM-2** in the NSW sample. Here, $P = 0.00077$ compared with α' of 0.0008, with the heterozygote variance exceeding that of the homozygotes. Deleting the single smallest fish from the NSW sample of 169 gave $P = 0.044$, a nonsignificant answer. The second significant result was *ADA** in the CASC sample. Here, $P = 0.00023$, this time with the homozygote variance exceeding that of the heterozygotes. Here, deleting the three smallest fish yielded a nonsignificant variance comparison ($P = 0.0025$, more than $\alpha' = 0.0008$). Thus, these two significant results appear to reflect the high sensitivity of the *F*-test to individuals lying at the extremes of the distribution. A total of 36 cases showed homozygote length variance exceeding heterozygote length variance, while 30 cases gave the reverse result ($P > 0.05$). None of the 11 tests of pooled data was significant, and for seven loci, homozygote variances exceeded those of heterozygotes, while the reverse was true for four loci ($P > 0.05$).

TABLE 4. Analysis of the relationships between length and multilocus heterozygosity, *H*: regressions of individual length on individual multilocus heterozygosity.

		Regression			Adjusted
Site	*n*	Intercept	Coefficient	*P*	r^2
CASC	101	37.171	0.821	0.121	0.014
GAB	83	33.984	0.143	0.613	0.000
St. H	155	36.142	−0.044	0.777	0.000
MAAT	110	34.311	−0.157	0.597	0.000
NSW	160	32.089	−0.065	0.720	0.000
WA	95	37.653	0.907	0.001	0.095

These results argue against any general or systematic correlations between individual locus heterozygosity and length or variance of length.

The regressions of length on multilocus heterozygosity, *H*, were estimated separately for each of the six sites (Table 4). Five

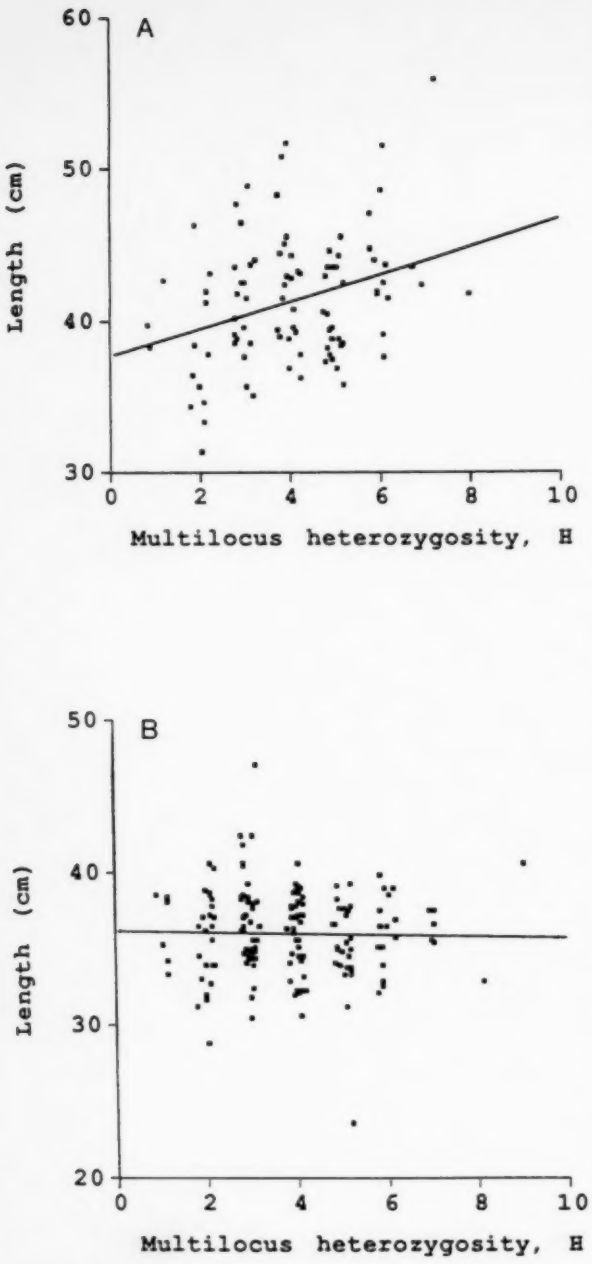


FIG. 2. Plots of individual length against individual multilocus heterozygosity, H, for (A) WA ($n = 95$) and (B) St. H ($n = 155$). Regression lines are shown (see Table 4 for parameters). Note that points are "jittered" to reduce overlap.

of the six sites showed no significant regression (three of these giving negative and two giving positive slopes), but for the WA collection, a significant positive regression was detected using α or α' levels of significance. It was this site that was responsible for the significant interaction term in the earlier analysis; excluding it renders the site by H interaction term nonsignificant ($P = 0.135$). For the WA fish, about 10% of the variation in length among individuals can be accounted for by variation in H. Pooling all sites gives a positive but nonsignificant regression coefficient ($b = 0.0187$, $P = 0.203$). Figure 2 illustrates the relationships between individual length and H for WA and for one of the sites with no correlation (St. H). WA was also the only population to show a significant correlation between H and length following a nonparametric (Spearman) test.

Another analysis considered whether fish falling into different H categories had different mean sizes or different coefficients of variation of length. Because the numbers of fish analysed at each of the six sites were not very large, for the correlation analysis, individuals with $H < 3$ were grouped together, as were those with $H > 5$. This gave five H categories. Not one of the 12 correlations of H category with length or CV was significant, and for both length and CV the number of positive correlations was similar to the number of negative correlations (Table 5).

Heterozygosity and meristics

Tests of homozygosity versus heterozygosity at each locus on the means or variances of the meristic characters are summarised in Table 6. Thirty-three tests (11 loci by three sites (CASC, GAB, MAAT)) were performed with respect to means and variances of each character for each locus (excepting the character ASY, which has a Poisson distribution and only means were considered). Thus the α' level used for significance testing for each meristic character was 0.0015 ($\alpha = 0.05$, $\alpha' = 0.05/33$). A total of 165 tests comparing means (33 tests for each of five characters) were carried out. Not one of these tests gave a significant result. In 79 cases, the homozygote mean exceeded the heterozygote mean, in 85 cases the reverse was true, and for one test the two means were equal. Of the 132 tests comparing variances (33 tests for each of four characters), 65 showed a greater homozygote variance and 64 a greater heterozygote variance. Nine of these variance ratio tests were significant, two where homozygote variances exceeded heterozygote variance and seven where the reverse was true. However, as pointed out earlier, the F-test is very sensitive to cases at the extremes of the distribution. For example, the five significant results for AR primarily achieve significance as a result of outliers. Two of these results are from the CASC collection, which become nonsignificant if the three fish at the extremes of the distribution are ignored, and two of the three significant AR results from MAAT become nonsignificant if the one fish with a fin ray count of 13 is ignored.

Analysis of pooled data gives similar results (Table 6). Here, for each character, 11 tests were carried out (one for each locus, giving $\alpha' = 0.05/11 = 0.0045$). Again, a few significant results were detected, but these were scattered among loci and were not consistent in direction. As with the preceding analysis, most of these significant results came from the variance comparisons and can be ascribed primarily to the effects of outliers.

The general conclusion is that there is no systematic relationship between individual locus homozygosity or heterozygosity and meristic character mean or variance in the orange roughy.

Not one of the 15 correlation coefficients (five characters, DR, AS, AR, LP, ASY, and three sites) between individual multilocus heterozygosity, H, and meristic counts was significant (results not tabled). Eight were positive and seven negative. None of the five correlations (five characters) for the pooled sample was significant. Three were positive and two negative.

Correlations were then analysed between H category (using five categories in the correlation analysis, $H < 3$, $H = 3$, $H = 4$, $H = 5$, $H > 5$) and the mean and CV of each meristic at each site.

TABLE 5. Correlations between multilocus heterozygosity category and the mean and coefficient of variation of length. Five H categories were used in the correlation analysis: $H < 3$, $H = 3$, $H = 4$, $H = 5$, and $H > 5$.

	CASC			GAB			St. H			MAAT			NSW			WA		
H	n	Mean length	CV	n	Mean length	CV	n	Mean length	CV	n	Mean length	CV	n	Mean length	CV	n	Mean length	CV
0	2	44.500	0.016										1	32.000				
1	4	35.000	0.403	4	30.750	0.199	6	36.167	0.069	3	34.667	0.148	4	34.250	0.037	3	39.667	0.052
2	8	33.875	0.376	15	34.867	0.106	24	35.500	0.083	17	32.294	0.132	22	32.773	0.111	12	38.000	0.112
3	19	42.316	0.134	16	34.438	0.139	39	36.744	0.090	28	34.214	0.130	28	31.120	0.087	19	41.316	0.091
4	29	39.759	0.194	19	36.000	0.104	37	35.946	0.072	31	34.806	0.134	44	31.227	0.093	22	42.273	0.096
5	19	42.316	0.174	15	32.867	0.113	27	35.074	0.084	15	33.267	0.119	39	32.051	0.082	22	40.364	0.075
6	18	41.222	0.156	8	35.875	0.118	15	36.133	0.072	11	33.091	0.145	22	31.318	0.138	6	43.692	0.088
7	2	44.000	0.129	5	34.000	0.091	5	36.400	0.031	4	30.500	0.172	3	37.000	0.094	3	47.667	0.152
8				1	36.000		1	33.000		1	33.000					1	41.000	
9							1	40.000										
r		0.658	−0.650		0.114	−0.791		−0.122	-0.519		−0.214	0.206		−0.713	0.499		0.782	−0.191
P		ns	ns		ns	ns		ns	ns		ns	ns		ns	ns		ns	ns

TABLE 6. Summary of relationships between individual locus homozygosity–heterozygosity and meristic characters. Values are numbers of cases where mean or variance of homozygotes (Ho) for each meristic character at each of the 11 loci exceeds the mean or variance of heterozygotes (Ht), and vice versa. The number of significant cases ($P < \alpha'$) is given in parentheses. As ASY has a Poisson distribution, variance comparisons are not given.

	Individual collections[a]				Pooled data[b]			
	Mean		Variance		Mean		Variance	
Character	Ho > Ht	Ht > Ho	Ho > Ht	Ht > Ho	Ho > Ht	Ht > Ho	Ho > Ht	Ht > Ho
DR	16 (0)	17 (0)	16 (0)	15 (0)[d]	4 (0)	7 (0)	6 (0)	5 (0)
AS	12 (0)	21 (0)	22 (2)	13 (1)	4 (0)	7 (0)	6 (0)	4 (1)[e]
AR	23 (0)	10 (0)	14 (0)	19 (5)	7 (0)	4 (0)	4 (1)	7 (4)
LP	10 (0)	22 (0)[c]	15 (0)	17 (1)[e]	3 (0)	8 (0)	5 (0)	6 (1)
ASY	18 (0)	15 (0)			6 (1)	5 (0)		
Totals	79 (0)	85 (0)[c]	67 (2)	64 (7)[f]	41 (1)	31 (0)	21 (1)	22 (6)[e]

[a]For each meristic character, $n = 33$ (three sites × 11 loci) and $\alpha' = 0.05/33 = 0.0015$.
[b]For each meristic character, $n = 11$ (11 loci) and $\alpha' = 0.05/11 = 0.0045$.
[c]One case where means were equal.
[d]Two cases where variances were equal.
[e]One case where variances were equal.
[f]Three cases where variances were equal.

TABLE 7. Correlations between multilocus heterozygosity category and the mean and coefficient of variation of meristic characters in pooled data. Seven H categories were used in the correlation analysis: $H < 2$, $H = 2$, $H = 3$, $H = 4$, $H = 5$, $H = 6$, and $H > 6$.

	DR			AS			AR			LP			ASY		
H	n	Mean	CV	n	Mean	CV	n	Mean	CV	n	Mean	CV	n	Mean	CV
0	2	18.500	0.038	2	3.000	0.000	2	11.000	0.000	2	19.000	0.000	2	0.000	—
1	11	17.636	0.046	11	2.909	0.104	11	11.182	0.036	11	19.000	0.024	11	0.364	1.387
2	40	17.900	0.043	40	2.925	0.091	40	11.075	0.038	40	18.800	0.037	40	0.325	1.617
3	61	17.951	0.037	62	2.871	0.118	62	11.065	0.032	63	19.016	0.035	63	0.333	1.616
4	78	17.782	0.040	78	2.872	0.117	78	11.103	0.043	79	19.051	0.037	79	0.342	1.615
5	49	17.878	0.045	49	2.939	0.108	49	11.122	0.060	48	19.063	0.037	48	0.271	1.825
6	37	18.054	0.037	37	2.919	0.095	37	10.892	0.056	37	19.000	0.041	37	0.270	1.880
7	11	17.818	0.023	11	3.000	0.000	11	10.818	0.056	11	19.182	0.031	11	0.455	2.055
8	2	18.000	0.079	2	3.000	0.000	2	11.500	0.061	2	19.500	0.036	2	0.000	—
r		0.363	-0.738		0.505	−0.544		−0.768	0.890		0.693	0.488		0.112	0.880
P		ns	ns		ns	ns		0.044	0.007		ns	ns		ns	0.009

This gave a total of 30 tests (mean or CV by each of five characters by three sites), not one of which was significant (results not tabled). Of the 15 correlations between H category and mean, eight were positive and seven negative whereas of the 15 tests between H category and CV, nine were positive and five negative. With respect to the pooled data (Table 7), which used seven categories in the correlation analysis ($H < 2$, $H = 2$, $H = 3$, $H = 4$, $H = 5$, $H = 6$, $H > 6$), none of the correlations involving

DR, AS, and LP was significant. The correlation between mean AR and *H* category was just significant ($P = 0.044$) with a Pearson test but not with the nonparametric Spearman test, while both tests gave a significant positive correlation between the CV of AR and *H* category. There was a significant increase in the CV of ASY with increasing *H* category.

Discussion

One of six southern Australian orange roughy fishery samples showed a significant, positive correlation between multi-locus heterozygosity, *H*, and length, and at this site (WA), 10 of the 11 polymorphic loci contributed to the correlation. However, while statistically significant, only about 10% of the variance in length could be attributed to the variation in *H*. Three of the remaining five sites showed negative and two showed positive correlations; none was significant. Pooling these five sites together, which were not significantly different from each other, gave a positive but nonsignificant correlation. None of the individual locus comparisons showed significant differences in mean lengths between homozygotes and heterozygotes, and numbers of cases where homozygotes exceeded heterozygotes were not significantly different from the reverse result.

We conclude that there is little evidence of any general correlation between heterozygosity and length in the orange roughy. The significant result for the WA site may well represent sampling error, although the probability of no correlation at this site was low (0.0014).

In New Zealand populations of orange roughy, which are allozymically very similar to Australian populations (Elliott and Ward 1992), a decline in heterozygosity has been recorded concomitant with increasing exploitation and declining biomass. It was suggested (Smith et al. 1990) that fishing activities were differentially removing the oldest and largest fish from virgin stocks and that these fish may have been more heterozygous than those that were smaller and younger. However, no data on the relationship between size or age and heterozygosity were presented. Our data do not suggest that larger fish are generally more heterozygous than smaller fish and therefore do not support the idea that the decline in heterozygosity noted in New Zealand stocks (primarily at the *GPI-1**, *GPI-2**, *LDH-1**, *MDH-1**, and *PGM-2** loci, all loci we have screened) resulted from selective trawling of larger, more heterozygous fish. However, neither is it possible to reject unequivocally such a hypothesis, as the sample sizes in the present study were such that a significant but small correlation would not have been detected. Given the intensity of fishing on the New Zealand grounds, even a very small correlation may be sufficient to ensure a decline in heterozygosity over time. The Australian populations were discovered and exploited well after the New Zealand populations, and we have examined the Australian populations at or shortly after the time of first exploitation. For example, the St. H sample we analysed was taken in 1990, 1 yr after this major spawning aggregation was first commercially targeted, and the WA sample was from the first commercial fishing of this species in this region. We intend to monitor these Australian populations for possible changes in heterozygosity as biomass declines.

There also appears to be very little association between heterozygosity, either at the individual locus or multilocus levels, and mean or variance of four fin ray characters and pectoral fin asymmetry. There was a very small number of statistically significant results from the plethora of tests undertaken, but these were inconsistent in direction and a majority of the significant variance ratio tests arose from the sensitivity of this test to outlying values. The two tests showing a significant correlation between multilocus heterozygosity category in the pooled data set and the coefficient of variation of LP and ASY were positive in value, indicating that, if anything, variance increased rather than decreased as heterozygosity increased.

In these analyses, the age structures of the roughy populations are not considered. Analysing cohort-specific correlations between morphological and genetic variables undoubtedly adds considerable power to such analyses. It enables, for example, possible effects of growth rate variance to be disentangled from genetic variance in survivorship. Furthermore, given that meristic variables such as fin ray counts are known to be at least partially dependent on environmental conditions (Taning 1952; MacGregor and MacCrimmon 1977), cohort-specific analysis removes or reduces the possible effects of between-year environmental variation on meristic counts. Despite such considerations, a sizeable proportion of past fish studies have either not separated individuals into age classes or have performed analyses on pooled data. Some of these studies have described relationships between morphology and genetic variables (Mitton 1978; Feder et al. 1984; Mitton and Odense 1985; King 1985); others have not (Yoshiyama and Sassaman 1983; Ryman et al. 1984).

Unfortunately, detailed age structured analyses are not possible for roughy populations. This is a slow-growing and long-lived species, maturing at between 20 and 30 yr of age, and perhaps living for 150 yr (Mace et al. 1990; Fenton et al. 1991). Allotting individual fish to particular cohorts cannot be done at present. Even if it were possible, given the probable age structures of samples of mature fish, it would be an enormous task to collect a sizeable number of specimens of any single cohort. While our data indicate that there is no overall correlation between length and heterozygosity, in the absence of age data, they do not allow us to say that there is no relationship between heterozygosity and growth rate, nor that there is no relationship between heterozygosity and survivorship. It could be, for example, that heterozygotes grow faster but die earlier than homozygotes, or vice versa. The most parsimonious explanation is that heterozygosity does not affect growth rates or survivorship, but it has been stated before that nature knows nothing of parsimony.

Our data also indicate that there is no relationship between heterozygosity and meristic variation. This conclusion seems fairly robust to the fact that year classes were not separated, for if heterozygotes of any particular year class were consistently less variable than homozygotes of that year class, then even if mean meristic values did fluctuate from year to year in accord with environmental fluctuations, data pooled across year classes should still show heterozygotes to be less variable than homozygotes.

We conclude therefore that our data provide little evidence for significant relationships between individual or multilocus heterozygosity and morphological variability in samples of orange roughy. Such associations seem to be more commonplace in hatchery strains of salmonid fishes (e.g., Leary et al. 1985; Danzmann et al. 1988; Ferguson 1992) than in large outbred populations of marine fish (see the introduction) and may reflect the undoubtedly smaller effective population sizes of hatchery fish. Linkage disequilibria and homozygosity for deleterious recessive alleles will be more pronounced in such populations, and therefore, these associations in hatchery salmonids may arise from associative overdominance rather than from the direct

effects of the screened loci. Further studies, preferably age structured, on species with highly divergent population sizes would be useful to support or refute such speculations.

Acknowledgements

We thank commercial operators for supplying fish and Mark Palmer for carrying out the meristic counts. We thank Peter Grewe, Tony Koslow, Roy Danzmann, and an anonymous referee for their comments on an earlier draft of this paper.

References

BEACHAM, T.D., AND R.E. WITHLER. 1985a. Heterozygosity and morphological variability of chum salmon (*Oncorhynchus keta*) in southern British Columbia. Heredity 54: 313–322.

BEACHAM, T.D., AND R.E. WITHLER. 1985b. Heterozygosity and morphological variability of pink salmon (*Oncorhynchus gorbuscha*) from southern British Columbia and Puget Sound. Can. J. Genet. Cytol. 27: 571– 579.

BEACHAM, T.D., AND R.E. WITHLER. 1987. Developmental stability and heterozygosity in chum (*Oncorhynchus keta*) and pink (*Oncorhynchus gorbuscha*) salmon. Can. J. Zool. 65: 1823–1826.

CHAKRABORTY, R. 1981. The distribution of the number of heterozygous loci in an individual in natural populations. Genetics 98: 461–466.

CHAKRABORTY, R. 1987. Biochemical heterozygosity and phenotypic variability of polygenic traits. Heredity 59: 19–28.

DANZMANN, R.G., M.M. FERGUSON, AND F.W. ALLENDORF. 1988. Heterozygosity and components of fitness in rainbow trout. Biol. J. Linn. Soc. 33: 285–304.

ELLIOTT, N.G., AND R.D. WARD. 1992. Enzyme variation in orange roughy, *Hoplostethus atlanticus* (Pisces: Trachichthyidae), from southern Australian and New Zealand waters. Aust. J. Mar. Freshwater Res. 43: 1561–1571.

FEDER, J.L., M.H. SMITH, R.K. CHESSER, M.J.W. GODT, AND K. ASBURY. 1984. Biochemical genetics of mosquitofish. II. Demographic differentiation of populations in a thermally altered reservoir. Copeia 1984: 108– 119.

FENTON, G.E., S.A. SHORT, AND D.A. RITZ. 1991. Age determination of orange roughy, *Hoplostethus atlanticus* (Pisces: Trachichthyidae) using ^{210}Pb:^{226}Ra disequilibria. Mar. Biol. 109: 197–202.

FERGUSON, M.M. 1992. Enzyme heterozygosity and growth in rainbow trout: genetic and physiological explanations. Heredity 68: 115–122.

KING, D.P.F. 1985. Enzyme heterozygosity associated with anatomical character variance and growth in the herring (*Clupea harengus* L.). Heredity 54: 289–296.

LEARY, R.F., F.W. ALLENDORF, AND K.L. KNUDSEN. 1984. Superior developmental stability of heterozygotes at enzyme loci in salmonid fishes. Am. Nat. 124: 540–551.

LEARY, R.F., F.W. ALLENDORF, AND K.L. KNUDSEN. 1985. Developmental instability as an indicator of reduced genetic variation in hatchery trout. Trans. Am. Fish. Soc. 114: 230–235.

LERNER, I.M. 1954. Genetic homeostasis. Oliver and Boyd, London.

MACE, P.M., J.M. FENAUGHTY, R.P. COBURN, AND I.J. DOONAN. 1990. Growth and productivity of orange roughy (*Hoplostethus atlanticus*) on the north Chatham Rise. N.Z. J. Mar. Freshwater Res. 24: 105–119.

MACGREGOR, R.B., AND H.R. MACCRIMMON. 1977. Evidence of genetic and environmental influences on meristic variation in the rainbow trout, *Salmo gairdneri* Richardson. Environ. Biol. Fishes 2: 25–33.

MCANDREW, B.J., R.D. WARD, AND J.A. BEARDMORE. 1982. Lack of relationship between morphological variance and enzyme heterozygosity in the plaice, *Pleuronectes platessa*. Heredity 48: 117–125.

MCANDREW, B.J., R.D. WARD, AND J.A. BEARDMORE. 1986. Growth rate and heterozygosity in the plaice, *Pleuronectes platessa*. Heredity 57: 171– 180.

MILLER, R.G. 1980. Simultaneous statistical inference. Springer-Verlag, New York, N.Y.

MITTON, J.B. 1978. Relationship between heterozygosity for enzyme loci and variation of morphological characters in natural populations. Nature (Lond.) 273: 661–662.

MITTON, J.B., AND M.C. GRANT. 1984. Associations among protein heterozygosity, growth rate, and developmental homeostasis. Annu. Rev. Ecol. Syst. 15: 479–499.

MITTON, J.B., AND P.H. ODENSE. 1985. Muscle esterase variation and size variation in the sand launce *Ammodytes dubius*. Mar. Biol. 87: 279–283.

MITTON, J.B., AND B.A. PIERCE. 1980. The distribution of individual heterozygosity in natural populations. Genetics 95: 1043–1054.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S.A. 70: 3321–3323.

RYMAN, N., U. LAGERCRANTZ, L. ANDERSSON, R. CHAKRABORTY, AND R. ROSENBERG. 1984. Lack of correspondence between genetic and morphologic variability patterns in Atlantic herring (*Clupea harengus*). Heredity 53: 687–704.

SHAKLEE, J.B., F.W. ALLENDORF, D.C. MORIZOT, AND G.S. WHITT. 1990. Gene nomenclature for protein-coding loci in fish. Trans. Am. Fish. Soc. 119: 2–15.

SMITH, P.J., R.I.C.C. FRANCIS, AND M. MCVEAGH. 1990. Loss of genetic diversity due to fishing pressure. Fish. Res. 10: 309–316.

TANING, A.V. 1952. Experimental study of meristic characters in fishes. Biol. Rev. Camb. Philos. Soc. 27: 169–193.

YOSHIYAMA, R.M., AND C. SASSAMAN. 1983. Morphological and allozymic variation in the stichaeid fish *Anoplarchus purpurescens*. Syst. Zool. 32: 52–71.

Spatial Model for the Population Dynamics and Exploitation of the Western Australian Rock Lobster, *Panulirus cygnus*

Carl J. Walters

Resource Ecology, University of British Columbia, Vancouver, BC V6T 1W5, Canada

and Norm Hall, Rhys Brown, and Chris Chubb

Western Australia Marine Research Laboratories, P.O. Box 20, North Beach, Western Australia 6020

Walters, C.J., N. Hall, R. Brown, and C. Chubb. 1993. Spatial model for the population dynamics and exploitation of the Western Australian rock lobster, *Panulirus cygnus*. Can. J. Fish. Aquat. Sci. 50: 1650–1662.

There is concern about whether long-standing regulatory measures (size and effort limits) are adequate to protect spawning stocks of the Western Australian rock lobster, *Panulirus cygnus*, and assure the highest average yields. Virtual population analysis and tagging studies indicate that exploitation rates are extremely high (possibly exceeding 70%/yr) on younger, mainly immature lobsters. To predict the efficacy of alternative regulatory schemes, it has been necessary to explicitly model the spatial and temporal dynamics of lobster abundance and fishing effort. Puerulus settlement (at age 9–11 mo) is mainly into shallow-water (<40 m) areas, and at 3–5 yr of age in most areas, there is a migration into deeper water where the animals mature after a further 1–2 yr. The size at migration (70–90 mm carapace length) ensures that at least some animals will have a chance to reach the breeding grounds offshore, where fishing effort has historically been lower than inshore, before they reach the minimum legal size (76 mm carapace length). By explicitly modelling the effort distribution as well as lobster movement and stock distribution, we hope to anticipate some consequences of changes in management policy that could not be predicted from biological assessments alone.

On s'inquiète pour la protection des stocks de géniteurs de la langouste d'Australie, *Panulirus cygnus*, par les vieilles mesures de réglementation (limites quant à la taille et à l'effort de capture). On se demande aussi si ces mesures peuvent maximiser les rendements moyens. Selon l'analyse des populations virtuelles et les études de marquage, les taux d'exploitation des jeunes langoustes, immatures surtout, sont extrêmement élevés (ils pourraient même dépasser 70 %/an). Pour prédire l'efficacité des règlements projetés comme solutions de rechange, il a fallu modéliser explicitement la dynamique spatio-temporelle de l'abondance et de l'effort de pêche de la langouste. Les puérulus s'établissent (à 9–11 mo) en eau peu profonde principalement (<40 m) et, à 3–5 ans, dans la plupart des endroits où ils se sont établis, ils migrent vers les eaux plus profondes où les langoustes arrivent à maturité après un délai d'encore 1 ou 2 ans. La taille à la migration (longueur de carapace de 70 à 90 mm) fait en sorte qu'au moins certains animaux auront une chance d'atteindre les zones de reproduction du large, où l'effort de pêche a toujours été inférieur à l'effort en eaux côtières, avant d'atteindre la taille minimale de capture autorisée (longueur de carapace de 76 mm). En modélisant explicitement la répartition de l'effort de même que le déplacement et la répartition des stocks de la langouste, nous espérons prévoir certaines conséquences d'un changement dans la politique d'aménagement, qui pourraient ne pas être prédites uniquement à partir des seules évaluations biologiques.

Received March 31, 1992
Accepted March 5, 1993
(JB455)

Reçu le 31 mars 1992
Accepté le 5 mars 1993

As Australia's largest single-species fishery, the western rock lobster, *Panulirus cygnus*, has been the focus of intensive research and management analyses over the past three decades. In 1963, it was one of the first major fisheries in the world to have a limited-entry policy for effort control (Bowen 1971; Morgan 1980; Hancock 1981). Since 1907, catches have been relatively stable at around 10 000 t, with an annual landed value of AUS$120–200 million. The main regulatory measures to avoid biological overfishing and promote economic efficiency have been the establishment of a minimum size limit (76 mm carapace length), the requirement to return ovigerous females to the sea, the setting of limits on the total number of lobster pots, and a seasonal closure from 1 July to 14 November. There have also been regulatory measures to avoid waste, including escape gaps that allow undersize lobsters to leave the pots, and delayed season openings in the Abrolhos Islands regulatory zone to avoid problems of handling newly moulted animals during the hot midsummer period. Fishers are allowed to fish in each of three regulatory zones (A, B, and C) along the Western Australian (WA) coast (Fig. 1).

The key policy issues at this time are whether the existing regulatory system will remain adequate to prevent overfishing in the face of increasing gear efficiency and fishing expertise and whether average yields and overall efficiency might be increased by altering key regulations such as the minimum size limit. To make credible assessments or predictions about these issues, careful examination of the spatial and temporal dynamics of both the stock and the fishing effort is required. There are four key stages in the life cycle of the western rock lobster: (1) the 9- to 11-mo planktonic larval period, where larvae from all along the

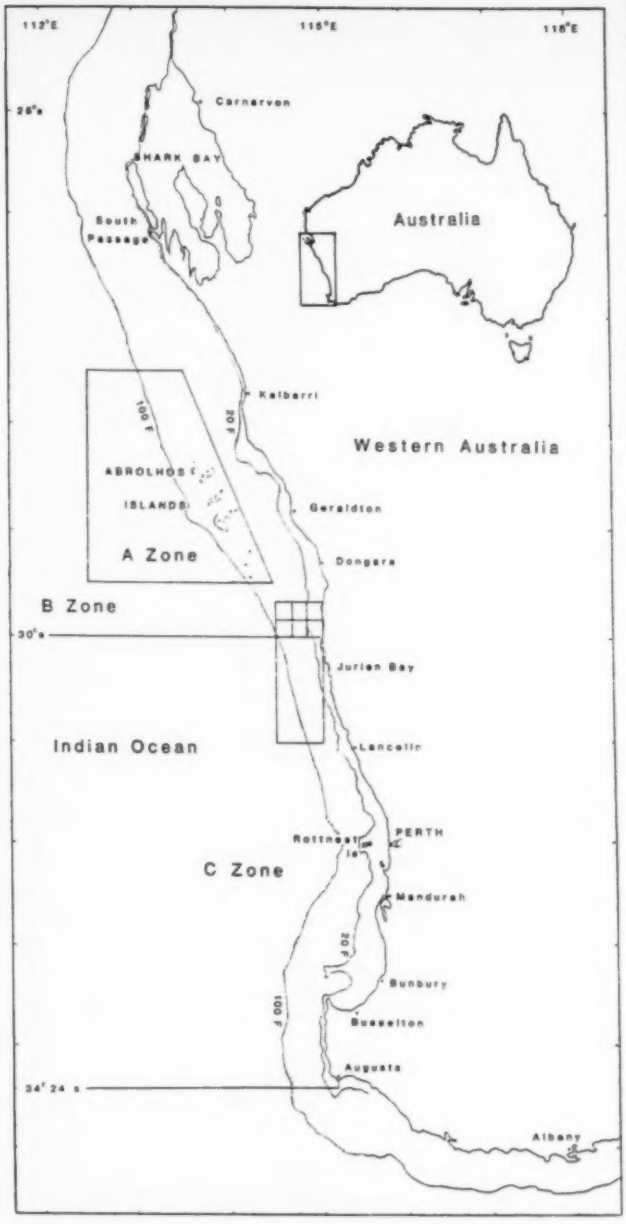


FIG. 1. Coast of Western Australia showing major ports and fishing zones for western rock lobster. The two different grid cell sizes used in the model (fast and slow versions) are shown on the 30°S latitude. Small cells are 16 × 16 km and larger cells are approximately 100 × 50 km.

coast are mixed and transported over large distances in the Indian Ocean (Phillips 1981); (2) puerulus settlement that occurs mainly in shallow waters, followed by 3–5 yr of inshore residence and growth (the majority of animals reach the 76-mm size limit during this period (Morgan et al. 1982)); (3) an offshore migration by 70–90 mm immature animals in a pale ("white") colour phase, taking place over a short period (2 mo) following a November–December moult, and involving very high vulnerability to fishing; and (4) maturation 1–2 yr after migration to contribute to the offshore spawning stock (Morgan et al. 1982). An exception to this pattern occurs in the Abrolhos Island area, where animals mature in shallow and deep areas at 60–70 mm carapace length, a year or two before becoming vulnerable to fishing.

At present, fishing is mainly concentrated on juvenile lobsters in the shallow-water areas and on whites migrating into deeper water. Less effort occurs in deeper water (where the spawning stock is concentrated), apparently due to the various returns, costs, risks, and discomfort of fishing further offshore. Fishers generally go out from port each day (or every few days when fishing is poor) to remove catches and redistribute pots. Lobsters are landed alive within a few hours after removal from the pots. Changes in fishing technology and/or regulations could substantially alter the fishers' incentives for targeting on shallow versus deep water, and hence, on exploitation patterns in relation to lobster size and maturity.

This paper describes a simulation model we have developed to help evaluate the possible consequences of altering size and effort limits and fishing season times. The model explicitly represents the spatial and temporal dynamics of the stock and of fishing effort by predicting abundance and effort distribution over a grid of spatial cells off the WA coast. We have tuned the parameters of the model to fit historical catch and effort data as closely as possible, and we compare policy alternatives by doing "might have" rather than "what if" comparisons. We also ask the model to predict what might have happened in the past, given historical patterns of disturbances such as oceanographic effects on larval settlement, if alternative policies had been followed. We believe that this is a safer and more credible use of the model, as compared with, for example, demanding that it make outright forecasts of future changes in the fishery. The model is programmed for a personal computer such that it facilitates rapid comparison of policy alternatives and testing of the sensitivity of its predictions to various uncertain parameters.

Model Structures and Assumptions

The simulation model consists of a series of submodels for updating population structure and the distribution of fishing effort over "fortnightly" (15-d) time steps, for up to 45 yr. The baseline starting simulation year is taken to be 1944 (1944–1945 fishing season), when catch and effort first began to increase rapidly. The simulation year commences in mid-November, when the annual rock lobster fishing season opens. The fortnightly time step is used for most calculations within each simulation year, rather than simpler equations integrating changes over an annual time step, in order to capture the seasonality of growth (moulting), recruitment, migration, closed fishing seasons, and redistribution of fishing effort in response to migration and seasonal depletion of more accessible (inshore) lobster concentrations.

The lobster population structure is represented as a size distribution (numbers by 4-mm length class, 60–100 + mm carapace length) in each of a series of rectangular spatial cells extending along the WA coast and offshore, from Shark Bay in the north to Augusta in the south (Fig. 1). The model can be run with various cell sizes (spatial resolutions), ranging from a detailed 250-cell version (16 × 16 km cells, Fig. 1) to a much simpler "fast" version with 12 cells each representing 1° latitude and 0.5° longitude (roughly 100 × 50 km, Fig. 1). Most of our

parameter estimation and gaming has been with the fast version, which requires only about 2 s of computing time for each simulation year (24 biweekly steps) on a personal computer with 20-MHz 80386 processor and 80387 math coprocessor. We have only used the detailed version to check the fast model results because the detailed calculations require about 12 s of computing time per year.

For each simulation year, the submodel calculations are organized to reflect the natural annual cycle of changes beginning in the spring (September–November). First, a spawning/recruitment submodel calculates aggregate egg production over the spatial grid (over the whole stock), and predicted total recruitment (to the 60-mm and larger size structure) 3 yr hence. Next, a set of submodels performs the following calculations for each of 24 fortnights within the year: (1) *moult animals* (grow them) into larger length classes; (2) apply natural mortality to all length classes; (3) add new recruits moulting into the 60–63 mm size class; (4) move some animals into a separate white (migrant) size structure, and revert postmigrant animals to the normal (red-sedentary) type; (5) predict total fishing effort for each regulatory zone and distribute this effort among the cells in the zone; (6) predict and remove catches and release mortalities (of undersize) from the length distributions (red and white); and (7) move white animals among cells to represent the offshore migration. Finally, the results of the fortnightly calculations (e.g., cumulative catches, efforts) are summarized for graphical display as the simulation proceeds. The following sections describe the various submodels in more detail.

Spawning/Recruitment

The stock and recruitment calculations involve three steps: (1) summation of annual egg production; (2) prediction of average recruitment (to 60 mm carapace length, at age 3) as a function of egg production (stock–recruitment relationship); and (3) adjustment for the deviation from the average recruitment using a time series of estimates of relative puerulus settlement, available for the period 1968–88. Steps (1) and (2) are typical of dynamic pool models in fisheries assessment; step (3) allows the model to reproduce historical stock dynamics more realistically and precisely, making the "might have" policy comparisons more credible.

The contribution of each spatial cell to egg production is estimated from the length distribution of lobsters in a cell by assuming that mean fecundity (proportion mature females times eggs per mature female times proportion female) is linearly related to body length above a threshold length at maturity (Morgan 1972; Chubb et al. 1989). There are large differences in the length at maturity between the Abrolhos Islands and Kalbarri (north coast) areas (60–70 mm) compared with more southerly areas (80 mm) (Chubb et al. 1989; Chubb 1993). These differences are represented by assigning each spatial cell to a "growth zone" where each zone is assigned a unique size at maturity and slope for the fecundity versus length relationship.

Given total egg production $E(t)$ for a year, recruitment of 60-mm lobsters in year $t + 3$, $R(t + 3)$, is predicted using the product of a Beverton–Holt stock–recruitment prediction $SsE/(1 + sE/R^*)$ and an "environmental effect" $P(t)^d$ to produce a modified Beverton–Holt recruitment equation of the form

$$R(t + 3) = \frac{SsE(t)\{P(t)^d\}}{(1 + sE(t)/R^*)}$$

where S is the survival rate from puerulus settlement to age 3, s is the maximum number of puerulus settling per egg $E(t)$, R^* is the maximum average recruitment rate of puerulus settling when $E(t)$ is large, $P(t)$ is an index of relative puerulus settle ment based on historical measurements, and d is a "density dependence" parameter measuring the strength of the regression relationship between historical puerulus settlement and subsequent recruitment to 60 mm carapace length. The index $P(t)$ is adjusted to have a mean of 1.0 over the historical data period (1968–88); d values below 1.0 imply that recruitment ends up being more than proportional to $P(t)$ for low settlement years ($P(t)$ below 1.0) and less than proportional for high settlement years, i.e., low settlements underestimate recruitment, while high settlements overestimate it.

The R^* and d parameters were estimated by "tuning" the model to historical data, in conjunction with the estimation of catchability (see Parameter estimation: Tuning the Model to Historical Data below). We took S to be $\exp(-3M)$, where $M = 0.23$ is the instantaneous annual natural mortality rate assumed for older animals (Morgan 1977; see next section). It is likely the natural mortality rate is higher for age 1–3 lobsters (Joll 1984), but we had no way to estimate that rate directly. However, this is not a critical issue because the key requirement is to estimate recruitment to age 3 when the animals first become vulnerable to pots. Provided s is sufficiently large, recruitment to age 3 will on average be equal to SR^*; when lower S values are assumed, the model-tuning procedure will simply assign higher values to R^* so as to ensure that the final recruitment is high enough to explain observed catches.

The really critical uncertainty in the recruitment calculation is about s. This parameter determines the risk of recruitment overfishing because sS represents the slope of the stock–recruitment relationship for low spawning stock sizes. There is no evidence in the historical puerulus settlement or catch data that there has been recruitment overfishing (Morgan 1977; Morgan et al. 1982). Environmental effects, in particular, water currents, probably play a major role in the level of puerulus settlement and hence recruitment to the fishery (Pearce and Phillips 1988; Caputi and Brown 1989; Pearce 1989). However, management measures, such as improved enforcement to prevent the sale of undersize lobsters and pot modifications to provide more escape gaps for small lobsters (Brown and Caputi 1986), could also be responsible for a modest increase in recruitment to the fishery over the past 20 yr. Lacking any evidence of overfishing, we chose to set $s = 0.0001$ (1 puerulus per 10 000 eggs). This is an optimistic assumption about the egg–puerulus survival rate; s would have to be an order of magnitude lower for the model to predict significant recruitment overfishing (50% reduction in recruitment, or greater) given our best estimates of historical stock size and exploitation rates. Values of s below about 0.5 times the assumed value would predict recruitment overfishing for policies that result in a decline of 50% or more in the spawning stock from its present level, which is not an unrealistic future scenario based on the current high exploitation estimate.

TABLE 1. Assumed proportions of rock lobsters moulting by season (indexed by month), size, and coastal region.

	Region											
	Southern				Abrolhos				Dongara			
Length (mm)	Nov.	Feb.	May	Aug.	Nov.	Feb.	May	Aug.	Nov.	Feb.	May	Aug.
60	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
64	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0
68	1.0	1.0	1.0	1.0	0.8	1.0	1.0	0.8	1.0	1.0	1.0	0.8
72	1.0	1.0	1.0	1.0	0.6	1.0	0.8	0.7	1.0	1.0	0.8	0.7
76	1.0	1.0	1.0	0.9	0.6	1.0	0.6	0.6	1.0	1.0	0.8	0.7
80	0.9	1.0	0.8	0.7	0.5	1.0	0.3	0.7	1.0	1.0	0.5	0.5
84	0.8	0.8	0.5	0.6	0.5	0.8	0.3	0.6	0.8	0.8	0.3	0.3
88	0.8	0.8	0.5	0.6	0.5	0.8	0.2	0.5	0.6	0.8	0.3	0.3
92	0.5	0.8	0.3	0.6	0.5	0.4	0.2	0.5	0.5	0.5	0.3	0.5
96	0.5	0.7	0.1	0.6	0.5	0.3	0.2	0.5	0.5	0.3	0.3	0.5
100+	0.5	0.3	0.1	0.6	0.4	0.1	0.1	0.5	0.5	0.1	0.1	0.6
Female length at maturity			80				60				80	
Slope fecundity versus length (1000 eggs/mm)			10.00				10.0				10.00	

Growth and Natural Mortality

Fortnightly growth and survival are modelled using the equations

$$N(L+1,i,j) = \{m(L,w)A(L,i,j) + [1-m(L+1,w)]A(L+1,i,j)Sw$$

$$N(1,i,j) = [1-m(1,i,j)]A(1,i,j)Sw + R(w,i,j)$$

where $A(L,i,j)$ is the number of lobsters in length class L in spatial cell i,j after fishing in any fortnight, $N(L,i,j)$ is the number in the length/cell before fishing for the next fortnight, $m(L,w)$ is the proportion moulting from length class L to $L+1$ in fortnight w, $Sw = \exp(-M/24)$ is the fortnightly natural survival rate, and $R(w,i,j)$ is the recruitment to length class 1 (60-63 mm carapace length) for cell i,j in fortnight w. The key assumption in this representation of growth is that animals either remain in a length class or moult into the next larger class. The average moult increment for western rock lobsters of 60–100 mm carapace length is around 4 mm in most areas (Chittleborough 1974, 1975, 1976; Chittleborough and Phillips 1975; Morgan 1977; Phillips et al. 1977, 1983; Joll 1984; Joll and Phillips 1984), so moving up two length classes per moult is unlikely (this is why we chose 4 mm as the length class width). In the Abrolhos Islands, mature females usually only grow about 2–3 mm per moult (Morgan 1977), which was represented by reducing the probability $m(L,w)$ of moulting to the next larger class for those cells i,j representing the Abrolhos area.

Our growth "model" is essentially the matrix $m(L,w)$ representing the probability of moulting (and moving up, on average, 4 mm in size) for animals of different sizes (L) in different fortnights (w). We constructed $m(L,w)$ tables (Table 1) by examining reported growth patterns based on laboratory and tagging studies (Chittleborough 1975, 1976; Chittleborough and Phillips 1975; Morgan 1977; Phillips et al. 1977, 1983; Joll 1984; Joll and Phillips 1984). For lobsters 60 mm and larger, moulting is concentrated in four periods, in the months of November, March, June, and August–September; to save com puter storage, we assumed that $m(L,w) = 0$ for all w except the four fortnights corresponding to the middle of these periods. Most animals except for breeding females are assumed to moult in the November period, and larger animals are assumed to miss more of the autumn and winter moults. The overall effect of the assumed moulting pattern is to produce a length–age pattern that looks much like a von Bertalanffy growth curve, with distributions of lengths at age around the curve that are roughly normal (unless grossly distorted by fishing) with coefficients of variation that are about 10% of the mean lengths at age.

The fortnightly recruitment rate $R(w,i,j)$ for cell i,j is calculated from the predicted total recruitment rate for the year, $R(t)$ (see above), by apportioning $R(t)$ among cells and fortnights as $R(w,i,j) = r(w)c(i,j)R(t)$. Here, $r(w)$ is the proportion of recruits reaching 60 mm caraparce length in fortnight w of their third year after settlement, and $c(i,j)$ is the proportion of the recruits that are assumed to have settled (and grown to 60 mm) in cell i,j. We assume that 0.4 of the recruits reach 60 mm in mid-November and 0.1 in each of mid-February, early March, early May, mid-May, early August, and mid-August, corresponding to the moulting times documented by Morgan (1977). Based on puerulus settlement rates on collectors scattered along the coast (Phillips and Hall 1978), we used two key assumptions to construct the assumed spatial distribution matrix, $c(i,j)$. First, we assume that $c(i,j)$ is normally distributed in the north–south direction, with a peak near the centre of the fishery (at Geraldton). Second, we assume that $c(i,j)$ decreases exponentially in the offshore (east–west) direction, except in the Abrolhos Islands area, so that 90% of the settlement will occur in the coastal and Abrolhos Islands cells.

Based on tagging data and size (grade) composition of catches, Morgan (1977) estimated the annual natural mortality rate M to be 0.23. We used this estimate without correction for size- and season-related (moulting, predation) variations that are likely present but cannot be estimated with any precision given the available data. In examining the sensitivity of the model, a higher

estimate of M (=0.5) was also tested.

White Moulting And Migration

The white migration (George 1958) involves a synchronous moult in late November (first simulated fortnight of the annual fishing season), by inshore animals mainly in the 70–90 mm size range (Fig. 2). While the duration of the subsequent white colour phase and migratory behaviour may differ considerably among individuals, the typical white is thought to be migratory and highly vulnerable to fishing for about 8 wk (four simulation fortnights). In the simulation, we assume that all whites appear in the first simulation fortnight and then revert to the red-sedentary type at the end of the fourth fortnight.

The number of whites of length L created in the first fortnight for cell i,j is taken to be $W(L,i,j) = w(L)N(L,i,j)$ for coastal cells i,j only (whites may be generated offshore, but are impossible to distinguish from the much larger pool of migrants coming from inshore). Crude estimates of the moulting proportions $w(L)$ by length were obtained in two steps. First, we did a length-based virtual population analysis (see Catch and Undersize Mortality) to estimate average total abundance by size for two representative fishing seasons (1983–84 and 1985-86). Second, we estimated the total number of whites initially present by size as the white catch (Fig. 2) divided by an assumed exploitation rate of 0.6 because fishing is intense and concentrated on the highly vulnerable whites (Hancock 1981; Bowen and Hancock 1989; Phillips and Brown 1989). $w(L)$ was then taken to be the number of whites initially present divided by the estimated total abundance less the number estimated to have turned white at smaller sizes. The results of this calculation are as follows:

Length (L)	68–71	72–75	76–79	80–84	84–87
w(L)	0.02	0.2	0.4	0.6	1.4

Thus, according to the method, it would appear that virtually all of the 84+ mm animals undergo the white moult; having more than 100% of the animals in the 84–87 mm class appear to moult is likely to be due to a significant proportion of larger animals undergoing a second white moult (George 1958). However, it may also be due to overestimation of the number of whites produced (assumed exploitation rate too low) or to underestimation of the number of larger animals present. Lacking better estimates of white moult rates from tagging data, we elected to assume that all inshore animals in the 84–87 mm category undergo the white moult.

For each of the four fortnights while whites are present, we assume that a proportion of the whites in each cell will move to adjacent cells to the west and to the north. The total proportion moving out of any cell depends on the cell size; for the detailed model (16 × 16 km cells), we took this proportion to be 0.8. For the larger, one-half-degree block cell size, the proportion was assumed to be 0.3. Of the proportion that move, we assume (from tagging studies) that 80% will go west and 20% north for small grid cells or 95, and 5%, respectively for the large cells (Phillips 1983). Based on tagging data, the average movement speed of migrating whites is about 1 km/d (14 km/fortnight).

Fishing Effort

Lobster licenses are issued to fish in two coastal zones and the Abrolhos Islands. Under current management regulations, there

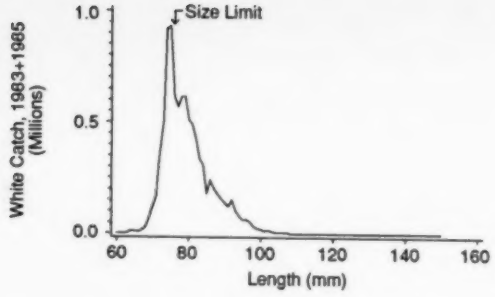


FIG. 2. Size distribution of white (migrant) phase rock lobsters estimated from catch monitoring samples for the 1986–88 fishing seasons.

is a limited transfer of pots between zones; therefore, we assume that the number of pots allowed to fish in each zone is set as a model policy variable. The Abrolhos (A) zone fishers are also allowed to fish in the adjacent coastal (B) zones from the coastal zone opening fortnight until the Abrolhos opening in mid-March. Prediction of fishing effort (pot lifts) for any fortnight for the cells within each zone is done in two steps: (1) the total number of pot lifts for the zone is predicted from the overall average catch rate (kilograms per pot lift); and (2) the proportion of these lifts occurring in each cell is predicted from the relative catch rate and distance offshore of the cell.

The number of pot lifts $L(z)$ for zone z in a fortnight is assumed to be $L(z) = P(z)\mathrm{Lm}/D(z)$ where $P(z)$ is the number of pots licensed for zone z, Lm is the maximum number of days fishing per pot per fortnight, and $D(z)$ is the mean number of days between pot lifts for the zone in that fortnight. Based on historical effort data for early season (peak) fishing times, Lm is taken to be 11 d (i.e., 22 d/mo). $D(z)$ is predicted from the expected catch rate ce(z) using the simple inverse relationship $D(z) = \mathrm{dl}/\mathrm{ce}(z)$ where dl = 1.0 is the average catch rate needed to induce the average fisherman to lift his pots daily. This rela tionship predicts that fishers will lift their pots progressively less often as the expected catch rate drops below dl. $D(z)$ is further constrained to be 1.0 or larger (i.e., no multiple pot lifts per day). This model for $L(z)$ results in the modelled fishery exerting around 60–70% of its maximum possible number of pot lifts in typical fishing years, with the maximum possible number of lifts per fortnight occurring only in the first few fortnights when catch rates are highest. The expected catch rate for the zone ce(z) is taken to be the simple average of the expected catch rates for the cells in the zone, where the expected rate for each cell for any fortnight is updated each year using the exponential averaging formula (new mean) = m (old mean) + $(1 - m)$ (catch rate for the fortnight this year) where the weighting factor m (a measure of fisherman memory) is assumed to be 0.5. Note that this averaging formula does not assume that fishers predict their catch rates from previous fortnights within the same fishing season. We trust the fishers to have the sophistication to look across years so as to correct for seasonal effects associated with typical depletion dynamics, moulting, and migration.

Effort for each zone, $E(z)$ is then distributed among cells using a simple gravity model similar to that used by Caddy (1975):

$$E(i,j) + E(z)a(i,j)/\mathrm{sum}\,A(i,j)$$

TABLE 2. Distribution of fishing effort and CPUE by depth for the WA rock lobster fishery, 1986-88 seasons.

Depth range (fathoms)	Fishing effort (million pot lifts)	% total effort	Catch CPUE	
			Million kg	kg/lift
0-10	6.62	60	4.31	0.65
10-20	1.69	15	1.25	0.74
20-30	1.51	14	1.31	0.87
30-40	0.40	4	0.47	1.18
40+	0.72	7	1.01	1.4

where $E(i,j)$ is the effort in cell i,j, $a(i,j)$ is an attractiveness index for cell i,j, and sum $A(i,j)$ is the sum of the attractiveness indices over the cells in the zone. The attractiveness index for each cell is calculated as $(ce(i,j)/o(i,j))^k$, where $ce(i,j)$ is the expected catch rate for the cell (see preceding paragraph), $o(i,j)$ is an index of the offshore distance of the cell (1.0 for coastal cells, 1.0 plus the number of cells away from coast times cell width in kilometres for offshore cells), and k is a "concentration parameter." High values of k (>>1.0) will cause effort to be concentrated in zones with the highest catch rate per distance travelled (measured by $ce(i,j)/o(i,j)$). For large values of k (>5.0) the model will approach the effort distribution pre dicted by the sequential effort allocation method of Hilborn and Walters (1987), i.e., for each fortnight the effort will all be placed in whichever cell has the highest attractiveness. For intermediate k values (1.0–5.0) the model will "smear" effort more broadly than the sequential method, thus recognizing variation among fishers in objectives, costs of operation in different cells, and information.

Logbook data do not permit detailed analysis of the effort distribution on a cell-by-cell basis except for our largest cell sizes, but there is good information on the depth distribution of effort (Table 2). For recent years, around 75% of the effort has been concentrated in shallow water (<20 fathoms, generally within model cells bordering the coast) where juveniles are abundant. About half of the effort in deeper waters (generally offshore) has been exerted during the white migration. We found by trial and error runs with the overall model that $k = 3.0–5.0$ would produce this general depth distribution; too much effort is distributed offshore after the white migration for $k < 3.0$, and too little effort is distributed offshore for much higher values of k.

The decision processes leading to onshore–offshore distribution of fishing effort are not well understood and represent a major area of uncertainty for the future of the fishery. If we have underestimated the extent of offshore movement of effort in response to declines in relative catch rates inshore (or improvements in technology for working offshore), we will in turn have underestimated the risk of overfishing on the spawning stock, which is largely concentrated in deep water (>20 fathoms) (Morgan et al. 1982).

Another possible weakness in our effort model is in the use of pot lifts as the unit of effort, rather than pot days or total soak time. We chose to use the pot lift measure because trapping efficiency is thought to decline rapidly after the first day in mulitple day sets in shallow water (<20 fathoms), so that the pot is fishing mainly on the first day of any set (or one day per lift). In experiments with single versus multiple day sets, N. Caputi and R. Brown (personal communication) found that only about 20% more lobsters are caught on the second and subsequent days of soaking in shallow water, due apparently to local depletion of residential lobsters in the neighbourhood of pots and to deterioration of baits.

Catch and Undersize Mortality

Catch of length L lobsters in cell i,j for any fortnight is predicted from the exploitation equation

$$C(L,i,j) = N(L,i,j)[1 - \exp\{-qv(L)E(i,j)\}]$$

where $C(L,i,j)$ is the catch, $N(L,i,j)$ is abundance (or $W(L,i,j)$ for whites when they are present), q is the overall catchability coefficient, $v(L)$ is a relative vulnerability for length L lobsters (whites of all lengths are assumed to have a single vulnerability vw), and $E(i,j)$ is predicted effort in the cell. This equation accounts for exploitation competition due to overlapping areas swept by pots when effort is high and differs from the standard fisheries catch equation only by ignoring natural mortality during the fortnight (accounted for in growth–survival equations; see above). For undersize lobsters, only a fraction (um) of $C(L,i,j)$ is removed from the simulated stock, representing post-release mortality. um is assumed to depend on the number of escape gaps required per pot, g, as $um = 0.213 \times \exp(-0.347\ g)$; prior to 1987 regulations ($g = 1$), this relationship results in um = 0.15 (Brown and Caputi 1986).

The catchability coefficient q is interpreted as the fraction of the fully vulnerable stock taken by one unit of fishing effort, where the fully vulnerable stock is defined as the size-colour groups with relative vulnerability $v(L) = 1.0$. We estimated q in conjunction with the recruitment parameters R^* and d by fitting the overall simulation model to time series of historical catch and catch per unit effort (CPUE) data. Physically, q can be defined as the area of detection by lobsters of a pot, times the proportion of lobsters in the detection area that will enter and remain in the pot, divided by the total area over which the lobsters are distributed within a cell. While the area of detection and probability of reaction could be assessed by localized tagging and behavioral studies, the total area over which lobsters are distributed would remain a gross unknown. Lobsters use only a small proportion of the ocean bottom (except during the white migration), concentrating on rocky bottom and reef areas that afford protection; although fishers may know most of these areas quite well, it would be difficult and expensive to map the areas with any precision. Thus, we had little choice but to estimate q directly from overall catch and effort data.

The relative vulnerabilities $v(L)$ were estimated by doing a length-based cohort analysis (Jones 1984) on length/frequency data for the total catch from two typical (average) fishing seasons, 1983–84 and 1985-86. The cohort analysis provides estimates of the annual number of lobsters entering each length class and the fishing mortality rate suffered by the class, given the length distribution of the catch, an estimate of the natural mortality rate, and estimates of the average time spent in each class (i.e., the growth rate). It is based on the catch equation

$$C(L) = F(L)[N(L) - N(L + 1)]/(F(L) + M)$$

where $C(L)$ is the catch of the length class, $F(L)$ is the fishing rate suffered by animals while passing through it, $N(L)$ is the number entering the class, and $N(L + 1)$ is given by

$$N(L + 1) = N(L)\exp\{-(F(L) + M)t(L)\}$$

where $t(L)$ is the average time spent in the class. Because $F(L)$ can be expressed as $F(L) = -\log\{N(L + 1) / N(L)\}/ t(L) - M$, the catch equation can be rewritten to express $N(L)$ as a transcendental function of $C(L)$, $N(L + 1)$, M, and $t(L)$. The cohort analysis proceeds by assigning a terminal population estimate $N(LL)$ to some large length class LL (we used the simple assumption that $N(LL) = C(LL) / 0.5$ for the 140–143 mm length class). Then, $N(LL - 1)$ is found by numerically solving the transcendental equation for it given $N(LL)$, using Newton–Raphson iteration. This procedure is repeated for $N(LL - 2)$ given $N(LL - 1)$, and so forth, until the abundance of the smallest length lobsters caught is estimated. For our analysis, we aggregated the length / frequency data into 4-mm "moult intervals" as used in the simulation (see Growth and Natural Mortality above), assumed $M = 0.23$, and estimated $t(L)$ from published data on the average number of moults per year for lobsters of different sizes (Chittleborough 1974, 1975, 1976; Morgan 1977; Phillips et al. 1977, 1983; Joll 1984; Joll and Phillips 1984). We calculated the average time per moult as $t(L) = 1/\text{nm}(L)$, where $\text{nm}(L)$ is the average number of moults per year. From the published growth and moulting data, we found that it is reasonable to assume that $\text{nm}(L) = 4(1 - L^*/140)$ where L^* is calculated from the length class index L by $L^* = 62 + 4(L - 1)$. Here, $L = 1, 2, \ldots$ is defined as follows: $L = 1$ corresponds to the 60–63 mm length class, $L = 2$ corresponds to 64–67 mm, and so forth.

The cohort analysis results (Fig. 3) indicate a striking pattern where the fishing rate is highest for animals in the 70–80 mm size range (but note that 70-75 mm animals are released, so that only a fraction will actually die). Smaller animals either do not enter, or escape from the pots through escape gaps (and between the pot battens or cane and sticks of other pots for very small animals). Larger animals may be subject to lower fishing rates for three reasons: (1) fishing effort is lower in the deeper waters where most of the larger animals are resident; (2) larger animals may be less active (feed less, be less prone to enter traps in search of food); and/or (3) larger animals may be distributed over a larger area of deepwater habitat, so that the proportion taken by (vulnerable to, exposed to, able to detect) any one pot lift is lower. There is anecdotal evidence that (3) is not the case and that there is in fact much less good habitat in deep water than in shallow water. The simplest explanation, and the one we assume in the simulation, is a combination of (1) and (2): larger animals are now subject to low fishing rates partly because fishing is concentrated elsewhere, and these large animals are also somewhat less vulnerable than smaller ones to effort placed in the depths (model cells) where they are concentrated.

Ordinarily the relative vulnerabilities $v(L)$ would be calculated from the cohort analysis results by taking ratios of $F(L)$ to the largest F (e.g., F for 72–75 mm in Fig. 3). Unfortunately this procedure does not provide a complete assessment for the rock lobster, since it does not provide a distinct estimate for the relative vulnerability (vw) of whites. Whites (pale-shelled migratory animals) make up 15% or more of the total catch, even though

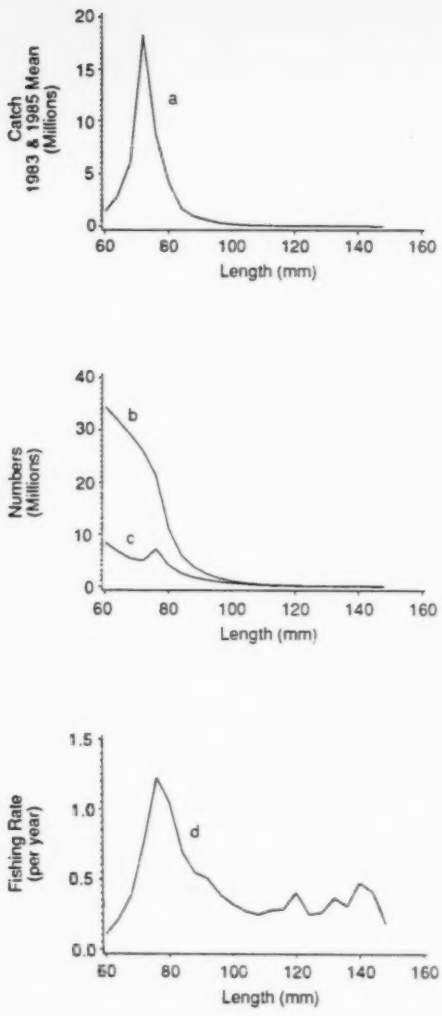


FIG. 3. (a) Size distribution of the 1986–88 rock lobster catch; (b) results of length-based cohort analysis to estimate numbers reaching each size interval per year; (c) average numbers at each size interval through the year (i.e., numbers reaching the size adjusted for the time to grow through the size interval and for total mortality during that period); (d) annual fishing rates by size interval.

they are present for only 8–10 wk of the fishing season. Further, fishers deliberately target them during their offshore migration, setting pots across presumed migration paths. Thus, there is reason to suspect that vw may be twice or more the peak $v(L)$ for nonmigratory red lobsters. Industry estimates of the "whites catch" (i.e., migrating animals) are often far higher than the 15% that is typical for monitoring data; this is because the industry classifies all lobsters caught over the main migration period, whether migrating or not, as white; using the industry estimates would result in even higher relative vulnerability estimates for the whites.

Lacking any direct way of estimating the ratio $\text{vw}/v(L)$, we proceeded by assuming that the exploitation rate for whites is

around 0.5 (Brown and Caputi 1983), implying that the number of whites initially present for each size class is about two times the measured catch and that the annual fishing mortality rate (F) for whites is around 5.0. When compared with the annual fishing rates for the remaining animals, and assuming that low fishing rates for larger animals are due partly to low effort in offshore waters, this assumption about the whites results in the following approximate relative vulnerability schedule:

Length class	60–63	64–67	68–71	72–75	76–79	79–81	82–85	86+	Whites
$v(L)$	0.03	0.06	0.13	0.40	0.40	0.30	0.20	0.20	1.00

Here, the estimates for 60–71 mm animals are likely to be quite accurate. The 0.4 values for 72–79 mm animals are purely a result of the assumed exploitation rate for whites. The values of 0.2 for larger animals are again somewhat speculative, being about half of what we would assume if the depth distribution of large and small animals were completely disjointed so that fishing rate differences could be ascribed solely to differences in effort (the fishing rate for older animals is about one quarter of the fishing rate for smaller ones, and about one quarter of the effort occurs offshore). Assuming that fishing rate F is proportional to effort, we would conclude that large and small animals are equally vulnerable to the effort directed at them.

Policy Options and Model User Interface

The simulation model is programmed to allow users to access several policy variables, and most of the population dynamics and effort parameters, through a user interface that presents the values in a spreadsheet format for easy editing and revision. A disk file system allows easy storage and retrieval of policy and parameter combinations, so that alternative scenarios can be constructed by adding new files or selecting among existing ones. A "script" file containing details of historical policy changes provides a simple method of incorporating these changes.

Policy variables accessible through the user interface are (1) minimum and (optionally) maximum size limits (by 4-mm carapace length increments); (2) required number of escape gaps; (3) a map defining the regulatory zone to which each cell is assigned; (4) the number of pots licensed to fish in each zone; and (5) a table of proportions of maximum effort allowed by fortnight in each zone (e.g, 0.0 represents having the zone closed for the fortnight, 1.0 represents having it open to fishing). Closed areas can be created by assigning cells to a new zone and setting the number of pots licensed to fish in this zone to 0.0.

Some other policy changes can be simulated by estimating (outside the model) the effects of these changes on population dynamics and effort parameters that are accessible through the user interface. For instance, changes in escape gap sizes can be simulated by changing the relative vulnerabilities $v(L)$. Policies that increase or decrease the cost of fishing (subsidies, taxes) can be represented through their effects on the dl parameter that defines the catch rate needed to induce fishers to make daily pot lifts.

The user interface is programmed so that policy and parameter changes can be introduced in any simulated fishing season (as well as before a simulation begins), simply by watching the simulation results as they appear graphically on the screen and touching the microcomputer keyboard when desired times for changes are reached. This allows the user to build up a complex scenario of changes, for example, progressive growth or decrease in the number of licensed pots over time. These changes may represent either a multiyear policy plan or uncontrolled dynamics that we have not thought to (or did not know how to) include in the original model.

The user interface also allows a number of options for graphical display of simulation results. As each simulation proceeds, the program displays crude maps of yearly changes in "juvenile" and "adult" abundance (coded by colour or grey scale intensity for each modelled spatial cell). Dynamic changes within each fishing season are displayed on similar maps showing fishing effort, CPUE, and catch for every fortnight. Besides the mapped information, the user can choose to display various graph "panes," where each pane shows a graph over years of one or a few key variables such as total catch, catch by processor grade, total fishing effort, and landed/export values of the catch. A few of these panes also display historical information (catch by processor grade, effort, CPUE) for comparison with simulated values.

Parameter Estimation: Tuning the Model to Historical Data

The user interface provides two options for tuning the model so as to provide parameter estimates that better fit the historical data. First, the user can simply run the model repeatedly with different parameter values entered through the spreadsheet displays and make visual comparisons between simulated and historical catches, efforts, and CPUEs. Second, the user interface provides access to a simple nonlinear estimation scheme for finding better estimates of a few aggregate (over all spatial areas) parameters (R^*, d, M, q). Generally we used trial and error simulations to obtain reasonable fits to the data and then used the nonlinear estimation to "fine tune" the estimates. The following section describes the nonlinear estimation scheme in more detail and presents general results of applying it to the model.

Estimation Procedures

Any dynamic model represents a mapping from a set of parameters (including initial values of variables) onto a set of predicted values, whether or not the model can be expressed as a simple equation reminiscent of some statistical regression formula. As noted by J. Schnute (personal communication), we may view any detailed simulation as a "stew" of calculations from which observable quantities (e.g., time series of catches) percolate to the surface for comparison with historical data. In this view, what nonlinear estimation does is to adjust one or more parameters of the stew so as to maximize (or minimize) some criterion for goodness of fit between the modelled observables and available data. Further, we need not regard all of the parameters in this stew as equally important or uncertain; like the model equation structure, some parameters may be treated as well determined by independent sources of information and argument (e.g., vulnerabilities $v(L)$, fixed from length composition data), so that the estimation algorithm can concentrate

on finding better values of a few key parameters of overall importance to model performance.

We choose to tune the model to the coastwide annual catches by processor grade (A, B, C+) and CPUE (kilograms per pot lift) for the fishing seasons 1976–77 to 1988-89. Processor grade A lobsters are mainly 76–78 mm carapace length (new recruits), grade B lobsters are 79–86 mm, and grade C and higher (C+) lobsters are mainly mature animals exceeding 87 mm carapace length. The catch grade data are thought to carry considerable information about the size/age composition of the stock, and hence about overall mortality rates. We used the data for recent fishing seasons only, so as to provide the best possible fit to current conditions and also to see whether the fitted model could accurately estimate earlier catches and catch rates.

Simple weighted least squares was used as the estimation criterion:

$$\text{SSW} = \text{sum over } i \ [1/\text{var}(i)\,][\text{yobs}(i) - \text{yhat}(i)\,]^2$$

where yobs(i) is the ith observation (catch or CPUE), yhat(i) is its predicted value from the simulation, and 1/var(i) is a weight defined by assigning a prior estimate of the relative variance var(i) expected for observations of type i. Expressing catches in millions of kilograms and CPUE in kilograms per pot lift resulted in most of the observed values being scaled to within the range 1.0–3.0, and we felt that there was no reason to assume heterogeneity of variance within this range; therefore for most estimation trials, we assumed var(i) = 1.0 for all i. We found that changing the weights had no noticeable (>1%) effect on the estimates.

Various algorithms can be used to minimize SSW. We have used the Simplex method recommended by Schnute (1982), the Fletcher–Powell algorithm (Press et al. 1986), and a Gauss method with step size corrections (Bard 1974, p. 111). By far the best choice has been the simple Gauss method. This method requires derivatives (sensitivities) of the predicted values with respect to the n parameters. These derivatives are easily obtained numerically by doing $n + 1$ simulations for each iteration in the nonlinear search; the first simulation provides base predictions, and for each of the other n simulations, one parameter is perturbed slightly (adding 0.0001) and the derivatives are calculated as the changes in predicted values divided by the perturbation. The step size correction procedure requires one to three additional simulations per iteration, to test for an "optimum" step length. We usually found that the Gauss iteration converged (parameters changing less than 0.001 per iteration) within 5–10 iterations, typically requiring a total of 30–40 simulations for three-parameter cases and about 1.5 h of microcomputer time. Two-parameter searches generally required less than 0.5 h. In contrast, the Simplex and Fletcher–Powell algorithms typically require 100–200 simulations (function evaluations) to satisfy the same convergence criterion.

Estimation Results

The reduced model (50 × 100 km grid cells) was able to reproduce recent historical catch and CPUE trends with considerable precision after only minor parameter tuning (Fig. 4). When the parameter estimates were substituted into the detailed (16 × 16 km grid) model, essentially the same fits were obtained except that predicted catch and CPUE were higher for early years.

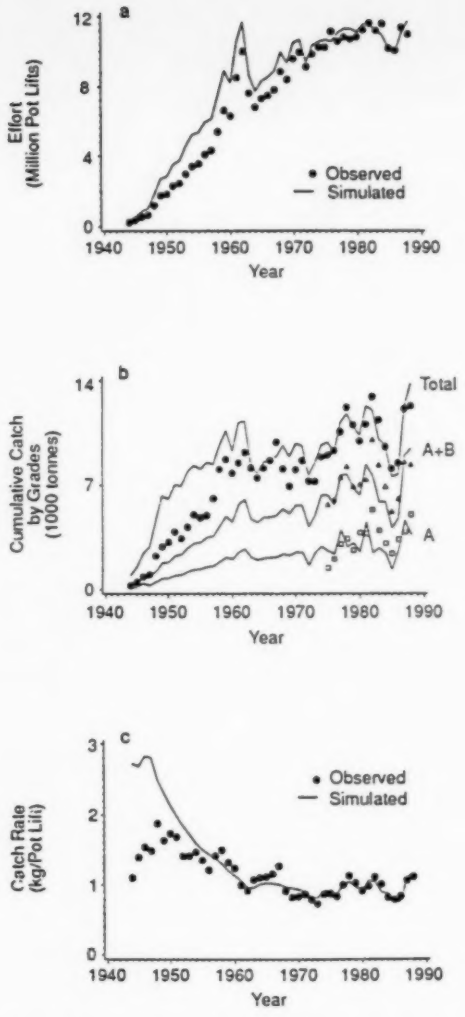


FIG. 4. Simulated versus observed fishing: (a) effort; (b) catch by processors category A (76–78 mm carapace length), B (79–86 mm carapace length), and C and higher (C+); (c) CPUE for the WA rock lobster fishery.

The power parameter d (for incorporating historical puerulus settlement variation into the predictions) was estimated to be 0.42, suggesting that puerulus settlement underestimates subsequent recruitment in poor settlement years and overestimates recruitment after good settlement years. For $d = 0.42$, the best estimates of the recruitment and catchability parameters were $R^* = 76.1$ and $q = 64.9$. However, we found that a higher d value (0.8) appears to result in a better visual fit to the data (model tracks strong changes in the 1980s better), although it results in a somewhat higher SSW (40.4 versus 25.9). We believe that the higher d value should be used for policy analysis because it is conservative in exaggerating recruitment variation and the possible management risks associated with that variation.

For $d = 0.8$, the best fitting estimate of average recruitment R^* was 76.9 million puerulus settling per year, or around 39 million recruits to the 60+ mm stock. This estimate is substantially higher than Morgan's (1977) estimate of 23 million annual

recruits to legal size but compares favourably with our estimate from the length-based virtual population analysis (VPA) of 34 million recruits to 60 mm.

The best estimate of q of 47.3 per million pot lifts (for $d = 0.8$) is also reasonable. This corresponds to a radius of attraction for each pot of around 274 m for white lobsters (173 m for 76–79 mm nonmigratory animals), if these lobsters were distributed over the entire area of each model grid cell (50 × 100 km) and every individual within the radius entered the trap. The estimated radius of attraction would be much less if we assume that only a small fraction of the area is actually used and fished (e.g., the radius would be 87 m if 10% of the area is suitable). These apparent attraction distances are well within the range observed or inferred from tagging studies and direct population estimates (Morgan 1977; Jernakoff 1987; Jernakoff and Phillips 1988).

Fishing effort calculated by the model for recent years underestimates the observed effort. Examination of logbook data suggests that the assumption of 22 d/mo of fishing is no longer valid. In recent years this has risen to 26 d/mo. Whether this is a response to increased competition among fishers or the implementation of new management strategies is uncertain.

The excellent agreement between model and recent catch data (Fig. 4) should not be interpreted as evidence that the model is structurally correct or that the parameter estimates are very accurate. Although some parameters may be treated as well determined from other sources, in reality, it is impossible to distinguish among a range of hypotheses about recruitment, catchability, natural mortality, and vulnerability of large lobsters. As the SSW values in the following table show, almost equally good fits are obtained when SSW is minimized over R^* and q (holding $d = 0.8$) for a range of assumptions about the relative vulnerability $v(L)$ of 84 + mm lobsters:

	Best fitting estimates		
Assumed vulnerability	R^*	q	SSW
0.1	103.1	30.7	39.0
0.2 (nominal)	76.9	47.3	40.4
0.4	71.9	54.9	46.2

Here the 0.4 case corresponds to assuming that all lobsters above the legal size limit are equally vulnerable. Note that there is a trade-off between R^* and q in this table: at one extreme (0.1 case), the stock may be quite large, be subject to relatively low fishing rates, and have relatively low adult vulnerability; at the other (0.4 case), the stock may be considerably smaller, be subject to very high fishing rates, and have low catches of grade C+ animals simply because very few animals survive to large sizes and there is little effort in deep water where they are concentrated. We can see no way to distinguish among these hypotheses with the data available. The uncertainty would not be resolved by fitting the model to much more detailed data (catch by cell/season) using a multiparameter estimation scheme that included the $v(L)$ and white moulting/movement rates as unknowns because the same basic arguments about trade-offs among the parameter estimates would hold at any scale of analysis.

When the stock assessment steps (VPA, vulnerability assessment, parameter estimation) were repeated with natural mortality rate $M = 0.5$ (rather than Morgan's (1977) $M = 0.23$), we obtained essentially as good a fit to the data as for the base line case. This higher M value is supported for early juveniles only by results in Joll (1984). Using the higher but still possible M has at least three major implications for the estimates and management predictions: (1) much higher estimates of average recruitment rate (over 300 million recruits to 60 mm carapace length per year, which appears unlikely) and lower estimates of catchability (q 20); (2) MSY estimated to be at least 50% higher than the recent average yield; and (3) much higher estimates of the rates of current to unfished spawning stock size (i.e., much less effect of the historical fishery on the spawning stock). For a further explanation of why implications (2) and (3) generally follow from increasing M in the assessment process, see Walters (1986, chap. 4). While uncertainty concerning M implies that there is a scenario that suggests an opportunity to substantially increase the yield from the fishery, we feel that it should be viewed instead as evidence that a key research priority should be to improve estimates of M and/or q through methods such as tagging studies.

With best fitting parameter estimates for recent years, the model generates higher CPUE and catch estimates for early years of the fishery than were actually observed (Fig. 4). The discrepancy can be interpreted as a "prediction" that modern fishers would be roughly 50% more successful than the early fishers (due to better boats and gear and knowledge of lobster distribution, for example) should a few modern fishers encounter the unfished abundance and lack of exploitation competition that fishers experienced in the 1940s. This difference in efficiency appears quite reasonable, in view of changes that have occurred in the distribution of fishing, vessel capabilities, pot hauling and handling methods, fish-finding equipment, and fishing knowledge. However, two other possibilities exist: (1) we may have overestimated the current catchability and exploitation rate, so that the stock available may not have declined as much as we have estimated (lower q and less decline relative to the unfished stock size would jointly contribute to make the model fit earlier years better); and (2) the natural mortality rate may have been higher (or recruitment rate lower), due to density dependence in the natural mortality rate under unfished conditions, so that the unfished stock was not so much larger than the present stock as the model predicts using Beverton–Holt type (essentially constant) recruitment and constant mortality rate.

The Model as a Management Game

The 45-yr simulations (fishing seasons from 1944–45 to 1988-89) require only about 2–3 min of microcomputer time for the reduced (50 × 100 km grid) model and about 12–15 min for the detailed model. A simulation is simple to interrupt at any time to alter policy variables and parameters. The graphics interface is programmed so that results from previous simulations can be left on the screen or restored from disk files and new results are then added as overlays permitting easy visual comparison among scenarios (Fig. 5). These combined features make the model easy to use as a game, where many management policies can be quickly tested and the results from each used to help decide what to try next. As promising options are uncovered, additional simulations can be easily made to check how robust the results are to uncertainties about various model parameters (e.g., a different size limit may initially look good, but this result may depend on the particular proportions assumed for white moulting/migration in relation to size).

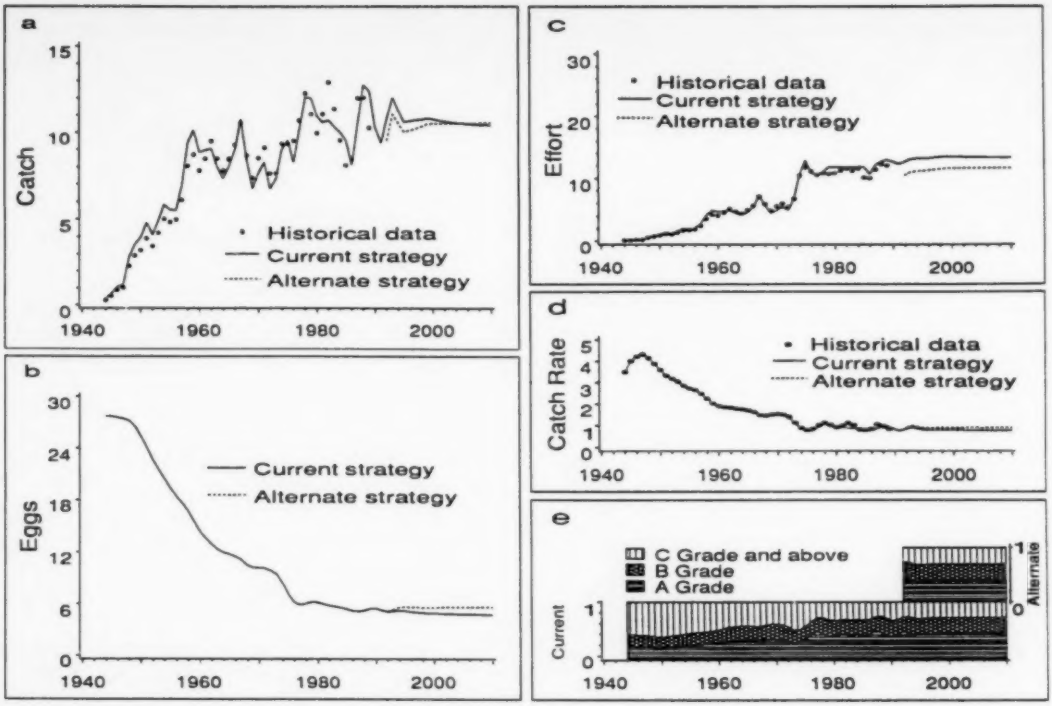


FIG. 5. Output from the most recent version of the model, comparing the current management strategy with an alternative strategy comprising a 1–30 June closure, a 10% reduction in the number of pots used, and a maximum carapace length of 116 mm for female rock lobsters. The alternative management strategy for this exercise was introduced in the 1992–93 season. Environmental variability for seasons after 1992–93 has been held constant.

The usefulness of the model and the gaming approach to assessing management policy changes has been tested in work shop sessions attended by both scientists, fishery managers, and fishing industry representatives with the practical experience of operating in the fishery. For example, we presented the model to the Western Australian Rock Lobster Industry Advisory Committee, a group of fishers (commercial and recreational), processors, and government fisheries officials who meet reguarly to review and recommend management policies. Members of the group suggested a variety of policy scenarios, which were then examined in the model. The predicted outcomes from the policy changes were judged by the fishers to represent the responses to the proposed management changes and likely catch outcomes. We had feared that the fishers would either be completely hostile towards the computer or uncritically accept its results. Instead we found them to be quite sophisticated in their appraisal of the model structure, and they carefully considered whether the possible weaknesses they had identified would affect the comparisons of the relative performance of alternative policy options. A useful outcome of the workshop testing of the model was that the rapid availability of the results helped to stimulate a highly focussed and productive debate among the committee members and a call for the continued development of the gaming approach.

The remainder of this section reviews some general conclusions that have emerged from gaming sessions to date about the efficacy of various changes in fishing regulations. Our general conclusion (using our best estimate of $M = 0.23$) has been that the fishery is now producing at near its maximum level in terms of both catch and export value of the catch, so that most policy changes would have a deleterious short-term effect, although they might reduce the risk of long-term difficulties such as recuitment overfishing.

There has been debate over the years about the size limit of 76 mm carapace length. This limit has been in place since the turn of the century, when it was introduced to satisfy marketing demands (Hancock 1981). It is too low to prevent the capture of immature lobsters (except in the Abrolhos, where it gives significant protection) and perhaps too high to fetch the maximum possible price per kilogram. Small lobsters are preferred in the key Japanese market. On average, over the various export markets for 1988–89, the small grade A lobsters (350–460 g whole weight) fetched around $25/kg, while the larger C grades (570–690 g whole weight) brought only $20/kg (Monaghan 1989). Industry estimates that lobsters in the 72–76 mm size range (currently not legal) would sell for about $32/kg. Thus, it might be worthwhile to either increase the size limit so as to better protect breeders or reduce it to increase economic value. The model supports this view, but indicates that the current size limit may in fact be about the best balance between conservation of spawning stock and economic return. It predicts that an increased size limit (to 80 mm) would result in a slight decrease (5%) in average CPUE and catch (due to allowing growth to larger sizes), as much as a 22% increase in spawning (annual egg

production), but a decrease in landed (6%) and export (11%) values of the catch. A reduced size limit (to 72 mm) would result in slightly (1%) reduced CPUE and catch (due to taking more small lobsters, foregoing growth), as much as a 13% decrease in annual egg production, and an increase in export value (7%) which would be greater during years when very strong year classes enter the fishery. Thus, while changes in the size limit could generate substantial changes in the spawning stock, it is likely that there would be smaller (but significant) changes in economic value, acting in the opposite direction.

The model predicts that combining a reduced size limit (72 mm) with an upper size limit of around 96 mm (perhaps by reducing the size of the pot entry port) would reverse the deleterious effect on the spawning stock of the size limit reduction (resulting in approximately 25% spawning increase). This "slot" size policy would result in the total catch being about 13% lower than at present in terms of weight and 4% lower in export value, unless the 72–76 mm lobsters could be sold at very high prices (>$40/kg). It is an interesting option for providing increased protection for the spawning stock while offering the fishers something in return (lower size limit) but does not address continuing increases in fishing efficiency that might erode any increase in spawning stock obtained initially.

Another key issue in recent years has been effort reduction by reducing the number of pots licensed to fish. The model predicts that pot reductions of 10% would be sufficient to cause noticeable (5% or more) decreases in annual fishing effort. With fewer pots fishing early in the season (when catch rates are high and all pots are lifted as often as possible), there would be a reduction in the exploitation rate of whites, and hence potentially an increase in the offshore spawning stock. However, lower effort early would mean more lobsters surviving to keep catch rates high later in the season, hence encouraging fishers to fish harder (continue making daily pot lifts) until later in the season and perhaps moving further offshore to deeper water to take the whites after they have reverted to the red type. Thus, a reduction in pot quota will result in only a modest reduction in catch or effort, which will be redistributed seasonally and spatially. The model estimates that the spawning stock would increase by approximately 7%, catch and export value would be reduced by about only 3%, and the effort by 7%, if the pot quota were reduced by 10%.

It has been suggested that recruitment to the offshore spawning stock might be improved and protected by opening the season later or by having a closure during the peak of the white migration. We found that a simulated closure for the month of December would indeed reduce the white catch greatly and result eventually in some increase (30%) in spawning. However, the main effects predicted for the policy would be to attract more fishing pressure to offshore areas during January–March and to shift the catch more to larger (less economically valuable) animals. Despite increased fishing effort later in the season, reduced vulnerability of the nonmigratory lobsters would prevent full recovery of the "slot" catch, resulting in a decrease in catch of about 16% and a decrease in export value of 19%.

It has been encouraging for model users to see the effects of alternative policy ideas, although often these ideas may result in negligible effects, or even the opposite effects than intended. But in each case, such model predictions have turned out to make good sense when the numerical and functional reasons for them have been examined. If nothing else, the model will certainly provide a moderating influence for government officials or industry representatives who believe that modest "tinkering" with the regulations can be of significant benefit.

Is the Rock Lobster Fishery in Danger?

Perhaps the most important issue in WA rock lobster management is whether current regulations are sufficient to ensure that the spawning stock size will remain high enough to produce adequate recruitment levels, given expected future improvements in efficiency of fishing. The modelling results (for M = 0.23) suggest that annual total egg production (combining spawner numbers and the size/fecundity relationship) has already been reduced to only 25–35% of its natural (unfished) level, and it would not be prudent to allow any further reduc tion. However, based on the possible (but less likely) modelling results for $M = 0.5$, the reduction in egg production is much less significant. Until this uncertainty associated with Morgan's (1977) best estimate of the parameter M is resolved, management decisions should be based on the more conservative and less risky assumption that $M = 0.23$.

The primary protection for the spawning stock now is apparently through the combined effect of four factors: (1) a significant percentage (33%) of the white migration consists of undersize lobsters that "escape" to deep water before being subject to legal fishing (Fig. 2); (2) effort is largely concentrated in shallow water (Table 2); (3) females in the Abrolhos Islands subpopulation mature at well below the legal size; and (4) q is lower in deep water and for larger animals. Immature lobsters reaching legal size in inshore waters are already subject to exploitation rates of at least 70% before moving offshore (Fig. 3), and increases in fishing effort or efficiency in inshore areas are unlikely to increase this rate dramatically (because of the exponential relationship between effort and exploitation rate, most additional effort would simply "sweep" areas already fished) unless the size limit is reduced.

Thus, the main concern should be whether exploitation rates will increase on postmigrant immature and mature lobsters in deep water. This could occur due to 1) an increase in pot siting efficiency (reduced proportion of pots set in poor habitats) by the fishers who currently work in deep water as more fishers learn where and how to fish these deeper areas by using better locational aids (e.g., global positioning systems), and/or (2) fishers encountering declining catch rates inshore due to poor recruitment years or increased competition from other fishers and hence moving off shore. These mechanisms involve changes in catchability coefficients; such changes are not included in the model and are notoriously difficult to predict because they involve human learning and inventiveness.

There are a number of possible management strategies for dealing with the possibility of increasing catchability coefficients and effort in deeper waters, assuming that these increases are not (even in principle) predictable in advance. One is a "passively adaptive" (Walters 1986) strategy of trying to monitor deepwater abundance more closely (e.g. spawning abundance indices) and then applying additional fishing restrictions (e.g., pot quota reductions, maximum size limit) when and if necessary. The second strategy is to implement a "robust" package of regulations that will protect the spawning stock no matter what happens to deepwater catchabilities; such a package might include closures to fishing in waters deeper than 20 fathoms, reducing pot neck (entry port) sizes so that 90–100+ mm animals cannot enter pots, and/or a simple maximum size limit. Note that

the robust policy would *not* include measures, such as a closure during the white migration, that might end up attracting more fishing effort offshore or otherwise simply redistribute the timing of effort movement. A third option not currently addressed by this model is the introduction of individual transferable quotas and a total allowable catch.

Any robust policy for spawning stock protection will inevitably involve some immediate loss in catch and fishing opportunity as the "price" to be paid for reducing the risk of long-term overfishing. This is without any guarantee whatsoever that the risk is even significant (it may even be that the spawning stock could be substantially reduced without impairing recruitment). Therefore, it becomes a purely political decision about whether the price, which we estimate from the model would probably be around 10% of the current landed value of the catch, is worth paying.

Acknowledgements

We thank our colleagues at the Western Australian Marine Research Laboratories for critically reading the manuscript and offering many helpful suggestions.

References

BARD, Y. 1974. Nonlinear parameter estimation. Academic Press, New York, N.Y. 341 p.

BOWEN, B.K. 1971. Management of the western rock lobster (*Panulirus longipes cygnus* George). Proc. Indo-Pac. Fish Counc. 14(II): 139–153.

BOWEN, B.K., AND D.A. HANCOCK. 1989. Effort limitation in the Australian rock lobster fisheries, p. 375–393. *In* J.F. Caddy [ed.] Marine invertebrate fisheries: their assessment and management. John Wiley & Sons, Inc., New York, N.Y.

BROWN, R.S., AND N. CAPUTI. 1983. Factors affecting the recapture of undersize western rock lobster *Panulirus cygnus* George returned by fishermen to the sea. Fish. Res. 2: 103–128.

BROWN, R.S., AND N. CAPUTI. 1986. Conservation of recruitment of the western rock lobster (*Panulirus cygnus*) by improving survival and growth of undersize rock lobsters captured and returned by fishermen to the sea. Can. J. Fish. Aquat. Sci. 43: 2236–2242.

CADDY, J.F. 1975. Spatial model for an exploited shellfish population, and its application to the Georges Bank scallop fishery. J. Fish. Res. Board Can. 32: 1305-1328.

CAPUTI, N., AND R.S. BROWN. 1989. The effect of environmental factors and spawning stock on the puerulus settlement of the western rock lobster. *In* B.F. Phillips. [ed.] Workshop on Rock Lobster Ecology and Management. CSIRO Mar. Lab. Rep. 207: 29 p.

CHITTLEBOROUGH, R.G. 1974. Western rock lobster reared to maturity. Aust. J. Mar. Freshwater Res. 25: 221–225.

CHITTLEBOROUGH, R.G. 1975. Environmental factors affecting growth and survival of juvenile western rock lobsters *Panulirus longipes* (Milne-Edwards). Aust. J. Mar. Freshwater Res. 26: 177–196.

CHITTLEBOROUGH, R.G. 1976. Growth of juvenile *Panulirus longipes cygnus* George on coastal reefs compared to those reared under optimal environmental conditions. Aust. J. Mar. Freshwater Res. 27: 279–295.

CHITTLEBOROUGH, R.G, AND B.F. PHILLIPS. 1975. Fluctuations of year class strength and recruitment in the western rock lobster *Panulirus longipes* (Milne-Edwards). Aust. J. Mar. Freshwater Res. 26: 317–328.

CHUBB, C.F. 1993. A study of the spawning stock of the western rock lobster. International Workshop on Lobster Ecology and Fisheries, 12–15 June 1990, Havana, Cuba.

CHUBB, C.F., C. DIBDEN, AND K. ELLARD. 1989. Studies on the breeding stock of the western rock lobster, *Panulirus cygnus*, in relation to stock and recruitment. FIRTA Project 85/57, Final Report.

GEORGE, R.W. 1958. The status of the white crayfish in Western Australia. Aust. J. Mar. Freshwater Res. 9: 537–545.

HANCOCK, D.A. 1981. Research for management of the rock lobster fishery of Western Australia. Proc. Gulf Caribb. Fish. Inst. 33: 207–229.

HILBORN, R., AND C.J. WALTERS. 1987. A general model for simulation of stock and fleet dynamics in spatially heterogeneous environments. Can. J. Fish. Aquat. Sci. 44: 1366–1369.

JERNAKOFF, P. 1987. An electromagnetic tracking system for use in shallow water. J. Exp. Mar. Biol. Ecol. 113: 124–144.

JERNAKOFF, P., AND B.F. PHILLIPS. 1988. Effect of a baited trap on the foraging movements of juvenile western rock lobsters. Aust. J. Mar. Freshwater Res. 39: 185–192.

JOLL, L.M. 1984. Natural diet and growth of juvenile western rock lobster, *Panulirus cygnus* George. Ph.D. dissertation, University of Western Australia, Nedlands, Western Australia. 194 p.

JOLL, L.M., AND B.F. PHILLIPS. 1984. Natural diet and growth of juvenile western rock lobsters *Panulirus cygnus* George. J. Exp. Biol. Ecol. 75: 145–169.

JONES, R. 1984. Assessing the effects of changes in exploitation pattern using length composition data (with notes on VPA and cohort analysis). FAO Fish. Tech. Pap. No. 256: 118 p.

MONAGHAN, P.J. 1989. Distribution and marketing of Western Australian rock lobster. Fish. Dep. West. Austr. Manage. Rep. 29: 125 p.

MORGAN, G.R. 1972. Fecundity in the western rock lobster *Panulirus longipes cygnus* (George) (Crustacea: Decapoda: Palinuridae). Aust. J. Mar. Fresh water Res. 23: 133–141.

MORGAN, G.R. 1977. Aspects of the population dynamics of the western rock lobster and their role in management. Ph.D. thesis, University of Western Australia, Nedlands, Western Australia. 341 p.

MORGAN, G.R. 1980. Population dynamics and management of the western rock lobster fishery. Mar. Policy 4: 52–60.

MORGAN, G.R., B.F. PHILLIPS, AND L.M. JOLL. 1982. Stock and recruitment relationships *in Panulirus cygnus*, the commercial rock (spiny) lobster of Western Australia. Fish. Bull. 80(3): 475–486.

PEARCE, A.F. 1989. The Leeuwin Current and the rock lobster. *In* B.F. Phillips [ed.] Workshop on Rock Lobster Ecology and Management. CSIRO Marine Laboratories Rep. 207: 29 p.

PEARCE, A.F., AND B.F. PHILLIPS. 1988. ENSO events, the Leeuwin Current and larval recruitment of the western rock lobster. J. Cons. Int. Explor. Mer 45: 13–21.

PHILLIPS, B.F. 1981. The circulation of the southeastern Indian Ocean and the planktonic life of the western rock lobster. Oceanogr. Mar. Biol. 19: 11–39.

PHILLIPS, B.F. 1983. Migrations of pre-adult western rock lobsters, *Panulirus cygnus*, in Western Australia. Mar. Biol. 76: 311–318.

PHILLIPS, B.F., AND R.S. BROWN. 1989. The Western Australian rock lobster fishery: research for management, p. 159–181. *In* J.F. Caddy [ed.] Marine invertebrate fisheries: their assessment and management. John Wiley and Sons, Inc., New York, N.Y.

PHILLIPS, B.F., AND N.G. HALL. 1978. Catches of puerulus larvae on collectors as a measure of natural settlement of the western rock lobster *Panulirus cygnus* George. CSIRO Rep. Div. Fish. Oceanogr. 98: 18 p.

PHILLIPS, B.F., N.A. CAMPBELL, AND W.A. REA. 1977. Laboratory growth of early juveniles of the western rock lobster *Panulirus longipes cygnus*. Mar. Biol. (Berl.) 39: 31–39.

PHILLIPS, B.F., L.M. JOLL, R.L. SANDLAND, AND D.W. WRIGHT. 1983. Longivity, reproductive condition and growth of the western rock lobster *Panulirus cygnus* reared in aquaria. Aust. J. Mar. Freshwater Res. 34: 419– 429.

PRESS, W.H., B.P. FLANNERY, S.A. TEUKOLSKY, AND W.T. VETTERING. 1986. Numerical recipes, the art of scientific computing. Cambridge University Press, Cambridge, N.Y. 818 p.

SCHNUTE, J. 1982. A manual for easy nonlinear parameter estimation in fishery research with interactive computer programs. Can. Tech. Rep. Fish. Aquat. Sci. 1140: 115 p.

WALTERS, C.J. 1986. Adaptive management of renewable resources. Macmillan, New York, N.Y. 374 p.

Characterizing In-stream Flow Refugia

Jill Lancaster and Alan G. Hildrew

School of Biological Sciences, Queen Mary and Westfield College, Mile End Road, London E1 4NS, England

Lancaster, J., and A.G. Hildrew. 1993. Characterizing in-stream flow refugia. Can. J. Fish. Aquat. Sci. 50: 1663–1675.

Flow in nine streams was examined in relation to refugia for invertebrates. Areas of bed maintaining low hydraulic stress throughout the discharge hydrograph could provide flow refugia for animals during spates. In one stream, near-bed shear stress and velocity were repeatedly measured in discrete patches. Three types were identified: "fast" patches maintained high hydraulic stress, "variable" patches showed the greatest change in stress, and "slow" patches maintained low stress and thus were potential refugia. Average stress increased with discharge, but potential refugia were prominent throughout and could be important for invertebrates. Abundances of refugia in eight comparison streams were characterized by changes in the frequency distribution of flow forces with discharge. Three stream types were identified that did not simply reflect channel size or morphology. Such stream-specific flow patterns could affect the structure of comunities through the differing provision of refugia. Longitudinal transport processes in these streams were investigated by solute dilution experiments and by the application of a model to measure an aggregated "dead zone". Transport (and dead zone volume) varied among streams and further reflected their refuge potential. Clearly, flow habitat features could intervene in population and community dynamics by providing refuge from spates.

On a examiné le débit de neuf cours d'eau susceptibles de servir de refuges aux invertébrés. Les parties du lit où les contraintes hydrauliques restent faibles durant tout l'hydrogramme pourraient servir de refuges aux animaux durant les crues. Dans un cours d'eau, on a mesuré à plusieurs reprises la cission et la vitesse du courant sur des étendues finies (plages) du lit. On a identifié trois types de ces plages : celles où la contrainte hydraulique est forte, celles ou cette contrainte est très variable et celles où la contrainte hydraulique est faible et qui, elles, pourraient servir de refuges. Les contraintes moyennes augmentent avec le débit, mais les refuges potentiels, notables partout, pourraient être importants pour les invertébrés. L'abondance de refuges dans huit cours d'eau de comparaison a été caractérisée par les changements de la distribution de fréquence des forces d'écoulement selon le débit. Trois types de cours d'eau ont été identifiés, mais pas simplement en fonction des dimensions du chenal ou de sa forme. Le diagramme d'écoulement propre au cours d'eau pourrait affecter la structure des communautés en influant sur le nombre de refuges possibles. Au moyen d'expériences de dilution de solutés et par l'application d'un modèle pour mesurer une «zone morte» globale, on a examiné les processus de transport longitudinal dans ces cours d'eau. Le transport et le volume de la zone morte variaient d'un cours d'eau à l'autre et ils en reflétaient davantage les potentialités à titre de refuges. De toute évidence, les caractéristiques hydrodynamiques des habitats pourraient influer sur la dynamique des populations et des communautés en fournissant des refuges contre les crues.

Received September 1, 1992
Accepted February 26, 1993
(JB615)

Reçu le 1 septembre 1992
Accepté le 26 février 1993

The importance of physical habitat to ecological processes is attracting renewed interest from lotic ecologists (Poff and Ward 1990; Hildrew and Giller 1994). Southwood (1977, 1988) proposed that habitat is a templet with axes related to life history strategies and other ecological responses of organisms, yet some fundamental features of the habitat structure of stream invertebrates remain poorly understood. Flow is a distinctive feature of rivers and streams, and it determines many of the solid and fluid physical structures of lotic habitats. Solid structures, such as channel form and substrate grain size, result from interactions between discharge, land form, and geology. Flow structure includes the complex spatial pattern of flow forces, such as shear and drag, produced at the water–solid interface as well as structures in the water column, including velocity profiles, vortices, and eddies. These flow characteristics can be examined over a range of spatial scales, at each of which different patterns may emerge. These scales range from patchiness in the thickness of the boundary layer (Carling 1992) to large-scale patterns in shear stress along whole river systems (Statzner and Higler 1986).

Flow habitat structure is not constant temporally, but varies with river discharge over time scales ranging from individual storm events to multiannual climate variations. Such fluctuations in flow are an obvious source of physical disturbance to stream communities. Unusually low flow may be accompanied by reduced oxygen concentrations, increased temperature, shrinking habitat, and desiccation, all of which can influence stream communities (e.g., Delucchi 1988, 1989). Unusually high flows are often accompanied by increased velocity and hydraulic forces on the stream bed, and these are the focus of the present investigation.

In the context of disturbance from high flows, we are concerned with two aspects of the interactions of flow with populations and community dynamics. Firstly, extreme hydraulic forces accompanying spates can erode animals from the stream bed, particularly where the substrate is moved. Secondly, flow

TABLE 1. Physical characteristics of the nine study streams.

Stream	Geographical coordinates	Distance from source (km)	Mean width (m)	Mean depth (cm)	Discharge ($m^3 \cdot s^{-1}$) Mean daily min.	Mean daily max.	Inst. max.
			Ashdown Forest, southeast England				
Broadstone Stream[a]	0°03′00"E, 51°04′30"N	0.42	1.38	14.22	0.001	0.11	0.20
Old Lodge[b]	0°04′26"E, 51°02′45"N	1.72	2.26	16.16	0.007	0.04	—
Lone Oak[b]	0°04′00"E, 51°04′36"N	1.82	1.55	12.30	0.001	0.05	—
Maresfield[b]	0°05′35"E, 50°59′45"N	5.29	2.42	17.80	0.017	0.30	—
Marsh Green[b]	0°04′45"E, 51°04′45"N	9.54	3.65	23.05	0.033	0.39	—
			Plynlimon, Mid-Wales				
Afon Gwy[c]	3°44′18"W, 52°27′25"N	3.25	3.08	18.20	0.010	0.615	2.33
Afon Hafren[c]	3°42′35"W, 52°28′25"N	3.30	3.15	21.16	0.023	1.667	4.58
			Rhinns of Kells, southwest Scotland				
Dargall Lane[d]	4°25′00"W, 55°04′30"N	2.25	2.75	24.48	0.002	1.70	4.66
Green Burn[d]	4°22′25"W, 55°04′40"N	2.00	1.88	20.02	0.001	1.49	3.41

[a]1991 discharge data from National Rivers Authority, Southern Region.
[b]Discharge data from Townsend and Hildrew (1983): underestimates of mean daily maximum discharge (see Table 3, especially Lone Oak and Old Lodge on 28 April 1992)
[c]1988 and 1990 discharge data from Institute of Hydrology.
[d]1985–90 discharge data from Solway River Purification Board.

structures in the water column determine the likelihood that entrained particles and benthic organisms will be transported out of the reach. Both processes, erosion and transport, are important determinants of population loss due to flow disturbance, and such losses are sometimes severe (e.g., Niemi et al. 1990; Giller et al. 1991). Many benthic communities are persistent, however, in that species composition and relative abundance remain much the same in the long term (e.g., Townsend et al. 1987; Boulton et al. 1992) and are resilient to short-term disturbances in that the original configuration is quickly reestablished after a disturbance event (e.g., Badri et al. 1987). The speed at which benthic communities can recover from short-term disturbances, without immigration from adjacent systems, is often much less than the generation time of the organisms. Such observations have led to the suggestion that there may be flow refugia in or associated with stream channels.

Refugia may influence populations and communities differently depending on the nature of the disturbance and its spatial and temporal scale (Sedell et al. 1990; Townsend and Hildrew 1994). Very low flows may act as disturbances, and refugia from such conditions may be important. This investigation, however, is concerned with refugia from disturbances created by high discharge events, of a magnitude likely to occur several times within the generation time of an organism. In this context, we define refugia as places not subjected to severe hydraulic stresses during such disturbances and thus likely to reduce density-independent mortality of benthic animals. During high discharge, the organisms most susceptible to high flow forces are most likley to use refugia. Spatially, these refugia probably occur at the scale of a channel subunit (features less than one channel width) or channel unit (Sedell et al. 1990).

The provision of flow refugia depends on environmental heterogeneity, such that patches are influenced differentially by the disturbance. During high discharge, individuals move, by passive or active means, into refugia where they avoid the disturbance and are subsequently available to recolonize denuded areas. There are three commonly cited categories of benthic flow refugia in running-water habitats (see also Sedell et al. 1990): (1) Animals may take refuge in a direction lateral to the bulk flow, i.e., over stream banks and in the flood plain. The flood plain can be an important refuge for fishes in lowland rivers (Schlosser 1991) and, although there is evidence that it may be important for some invertebrates in some circumstances (Badri et al. 1987), its overall importance as a refuge to macroinvertebrates is equivocal. (2) Animals may take refuge in the hyporheic zone, and this is perhaps the most frequently cited flow refuge. Although large numbers of invertebrates have been found in hyporheic zones (Stanford and Gaufin 1974; Williams and Hynes 1974; Williams 1984) and there is some evidence supporting the role of the hyporheic zone as a refuge for some organisms (Poole and Stewart 1976; Marmonier and Creuzé des Châtelliers 1991; Dole-Olivier and Marmonier 1992), there is also evidence that it may not be important in all situations (Giberson and Hall 1988; Palmer et al. 1992). (3) Animals may shelter in refugia in the stream channel itself, places that maintain low and/or invariable shear stress throughout the discharge hydrograph. Many streams have virtually no flood plain and a very limited hyporheos and, in such circumstances, in-stream flow refugia may be very important to the structure and function of the benthic macroinvertebrate community. Hitherto, this third category of refugia has received rather little attention and is the subject of the present investigation.

Stream macroinvertebrates are highly mobile and in a process of continuous redistribution (e.g., Townsend and Hildrew 1976). During field enclosure experiments, Lancaster et al. (1990) "fortuitously" acquired evidence that macroinvertebrates can accumulate in areas of locally reduced flow. The mesh-walled enclosures were permeable to most of the resident stream benthic macroinverterates, but flows were reduced inside the cages relative to the surrounding stream. The number of colonists

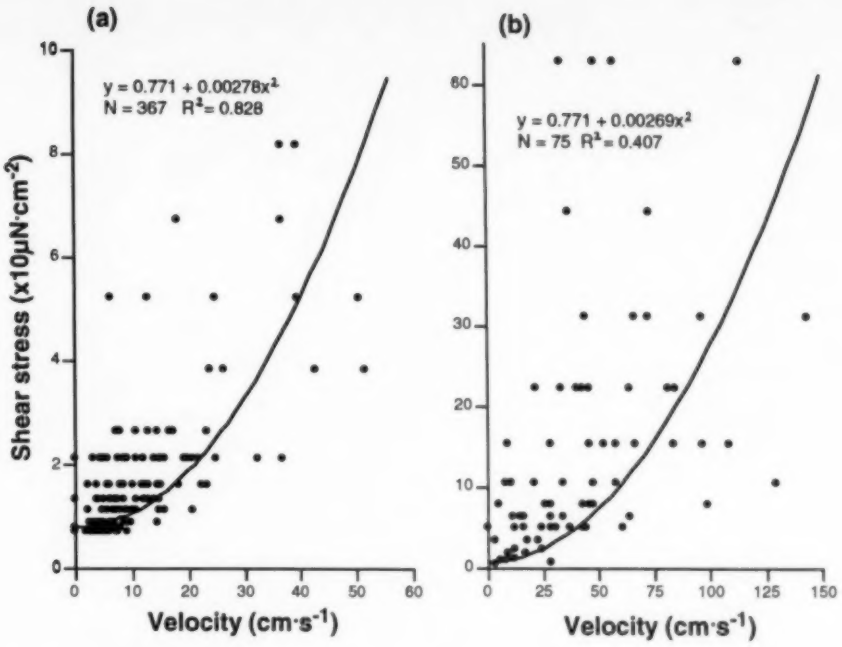


FIG. 1. Relationship between bottom shear stress and near-bed velocity in (a) Broadstone Stream at moderate discharge (0.006 $m^3 \cdot s^{-1}$, 15 May 1991) and (b) Dargall Lane at high discharge (0.543 $m^3 \cdot s^{-1}$, 11 April 1991). Note that axes scales differ. Equations for the lines were derived using nonlinear regression analysis.

inside cages during 24-h periods was correlated significantly with discharge, and this suggests that the cages acted as artificial flow refugia.

This study addresses three questions: (1) In natural channels, are there areas on the stream bed that maintain low hydraulic stress, even during spates, and that could thus act as in-stream flow refugia for stream animals? (2) Is it possible to quantify the abundance of flow refugia in a stream? (3) Are there differences in flow patterns and the relative abundance of in-stream flow refugia among stream channels that may be reflected by macroinvertebrate communities? Such questions are fraught with difficulty, for in choosing methods, we are faced with the conflicting demands of technical feasibility, relevance of the scale of measurement to the organisms, and the spatial and temporal extent of the required data set.

Study Sites

We chose a single channel for very intensive study and several comparative sites for less intensive investigation. Broadstone Stream was chosen for intensive investigation because its macroinvertebrate community is well known and persistent (Townsend et al. 1987) and because there is evidence of strong competitive interactions (Hildrew and Townsend 1980; Lancaster et al. 1988) and intense predator impacts (Hildrew and Townsend 1976; 1982; Lancaster et al. 1990; 1991) among its invertebrates. It is therefore hypothesized that in-stream flow refugia may be particularly important to the structure of this community. In addition to Broadstone Stream, we chose eight other channels for comparison, including upland and lowland streams, all of which are the subject of long-term investigations by our laboratory.

The nine streams (Table 1) were located in three regions of Great Britain: (1) the Ashdown Forest in East Sussex, southeast England, (2) Plynlimon in Powys, Mid-Wales, and (3) the Rhinns of Kells in Galloway, southwest Scotland. The Ashdown Forest and its lowland streams have been described previously (Townsend et al. 1983). The three small first-order streams, Broadstone Stream, Lone Oak, and Old Lodge, drain acidic heath and some mixed woodland; the two larger, higher order streams, Maresfield and Marsh Green, flow through mixed woodland and agricultural lands. In contrast, the stream sites in Wales and Scotland are typically torrential upland streams draining either moorland (Afon Gwy and Dargall Lane) or coniferous forest (Afon Hafren and Green Burn).

Methodological Approach

Hydraulic forces on stream substrates are extremely heterogeneous and there are many methodological problems of measuring such forces on a scale relevant to benthic invertebrates (Nowell and Jumars 1984; Davis and Barmuta 1989; Statzner and Müller 1989; Carling 1992). We did not seek to measure flow around individual organisms, but to examine larger scale flow habitat features that may have relevance to the population phenomena of distribution and abundance. We measured flow forces on many patches of stream bed and characterized hydraulic conditions on a reach scale by examining frequency distributions of such measurements.

Transient storage models of longitudinal transport processes were applied in order to (1) treat reach-scale transport characteristics as integrated measures of flow refugia and (2) demonstrate

TABLE 2. Summary of Broadstone Stream flow survey dates, discharge, and Froude number. Symbols and *N* values apply to Fig. 4: *N* values are for shear stress and velocity data sets, respectively. Velocity data were not available for 5 November 1990.

Date	Discharge ($m^3 \cdot s^{-1}$)	Fr	Symbol	*N*
5 November 1990	0.002	0.012	●	376
26 November 1990	0.005	0.012	○	355, 406
28 January 1991	0.005	0.019	◆	421, 544
4 February 1991	0.005	0.022	❑	422, 549
17 February 1991	0.012	0.048	✖	412, 537
7 March 1991	0.008	0.030	◊	433, 570
20 March 1991	0.020	0.069	▼	388, 584
15 May 1991	0.006	0.035	△	384, 550
11 June 1991	0.004	0.025	▲	354, 547
26 August 1991	0.001	0.0060	■	354, 543

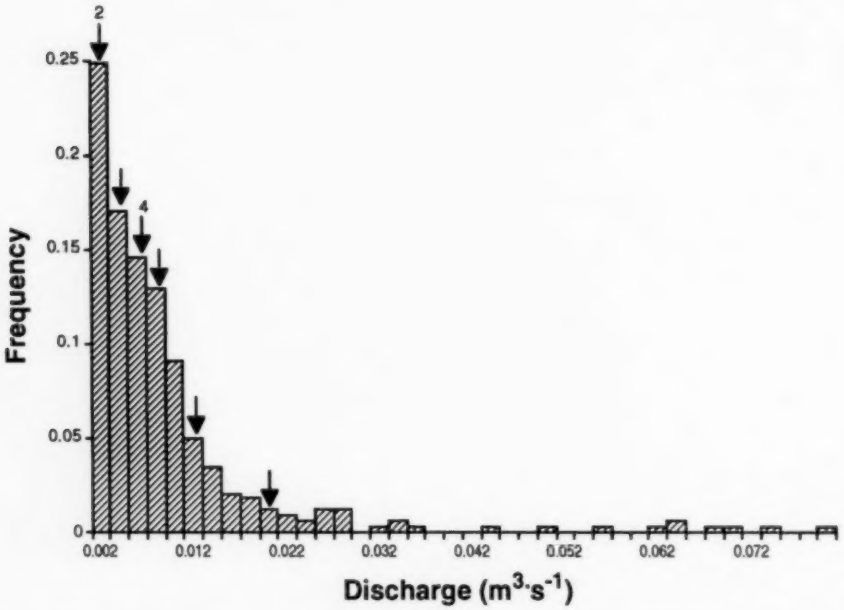


FIG. 2. Frequency distribution of mean daily discharge in Broadstone Stream over one calendar year, 1991. Arrows indicate discharges at which flow surveys were carried out. Numbers above arrows designate two or more surveys per discharge class.

differences in transport properties among streams, as possible measures of the likelihood that animals entrained in the water column will be transported out of the reach. Traditionally, the advection dispersion equation has been used to study transport processes (e.g., Fischer 1967). The limitations of this approach have been realized and the theory was modified more recently to include the effects of storage zones (Beer and Young 1983; Bencala and Walters 1983; Young and Wallis 1986; Wallis et al. 1989). Areas of transient storage, sometimes called hydraulic "dead zones", are places on the periphery of the main channel flow that are not part of the bulk flow but where some form of mixing or exchange occurs with the bulk flow. They are usually located in stream margins, turbulent eddies, wakes around roughness elements (e.g., boulders, logs), and reverse flows associated with pools and bends.

Reynolds (1988) was perhaps the first to recognize the importance of dead zones to organisms, particularly planktonic algae, living in the water column of rivers. The persistence of suspended algae in rivers is paradoxical without the existence of hydraulic dead zones in which seed populations can be maintained throughout the discharge hydrograph. Reynolds et al. (1991) showed that the distribution of phytoplankton in rivers tracks the velocity structure in river channels. Similar arguments can be applied to the larger macroinvertebrates inhabiting lotic systems. We hypothesized that the aggregated dead zone features of a reach are related to the abundance of in-stream flow refugia for macroinvertebrates.

Methods

Flow surveys were carried out on each stream at several different stages of discharge: measurements included stream width,

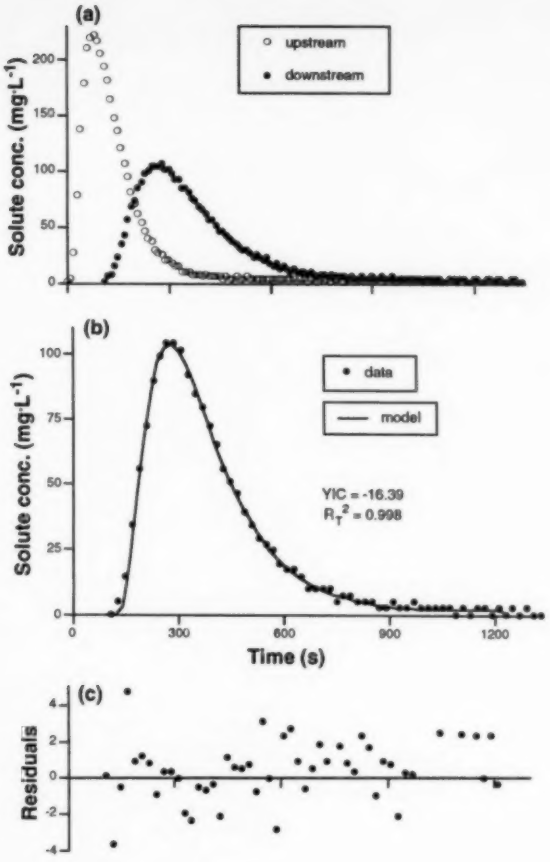


FIG. 3. Example of solute tracer data and ADZ-analysis: (a) raw data for a typical pair of concentration profiles at upstream and downstream probes; (b) model fit to raw data of downstream profile in Fig. 3a, with a statistically high coefficient of determination (R_T^2), and error variance norm (YIC); (c) residual errors between raw data and model in Fig. 3b.

depth, near-bed current velocity, and shear stress. Current velocity was measured 2–3 cm above the stream bed and averaged over 10–20 s using a "mini" bucket wheel velocity meter (5 cm in diameter) fitted with a photo-fibre-optic sensor to ensure accurate measurements at low flows. Shear stress at the substrate surface was estimated using Fliesswasserstammtisch (FST) hemispheres (Statzner and Müller 1989; Statzner et al. 1991), a series of hemispheres of varying density that are swept off the substrate at known shear stresses. Neither the hemispheres nor the bucket wheel are sensitive to the horizontal direction of flow. Shear stress at the substrate surface should be proportional to the square of velocity (Gordon et al. 1992). The relationships between bottom shear stress and near-bed velocity in a small lowland stream at moderate discharge (Broadstone Stream) (Fig. 1a) and a larger torrential upland stream at high discharge (Dargall Lane) (Fig. 1b) are representative of the results found in all streams on all occasions. Both regression equations are a significantly good fit to the data. The y-intercept of the regression equations is fixed by the detection limits of the equipment. The estimated slopes of the lines in Fig. 1 are similar and have overlapping 95% confidence limits ((a) 0.00257–0.00289, (b) 0.00189–0.00349). The degree of scatter is greatest, and hence R^2 values lowest, in Fig. 1b. This scatter reflects (1) the discontinuous nonlinear nature of shear stress as estimated by the FST hemispheres, (2) the practical difficulty of measuring hydraulic forces accurately when discharge and turbulence are high, and, perhaps, (3) buffeting of the bucket wheel by nonhorizontal currents in very turbulent places, hence contributing to measurement error (Gordon et al. 1992). Accurately measuring hydraulic forces on the stream bed of natural channels is extremely difficult and our methods are not without their limitations. Our objective, however, was to examine patterns within and among different channels, and for this purpose the methods are appropriate.

Surveys were carried out at 10 discharges in a 200-m stretch of Broadstone Stream (Table 2; Fig. 2). The discharge range was sufficiently large to describe adequately the patterns of hydraulic stress. We are confident that the frequency distributions of flows at the highest discharge measured (Fig. 2) are representative of those at even higher levels. Indeed, it would be impossible to survey flows at very high discharge owing to high turbidity and suspended sediment in this stream at such times. On 200 transects, 1 m apart, measurements were taken at the centre of the stream and at two points either side, equidistant from the edge and centre point, a total of 600 spots. Very shallow or deep water, or areas of extremely complex substrate architecture, precluded shear stress and velocity readings at some points, so the number of measurements is often less than the number of spots. Permanent markers on the stream bank enabled us to take readings at the same spots on each visit. The eight comparison streams were each surveyed four times, with 100 spots on a random grid and three or five measurements per transect (depending on stream width), the latter spaced at approximately two stream widths (Statzner and Müller 1989). Stream discharge was monitored continuously on five of the nine streams and data were processed by the various measuring authorities (see Table 1). These data were used to calculate mean reach Froude numbers, a dimensionless parameter combining variables that describe channel morphology (Newbury 1984), using the equation

$$\mathrm{Fr} = \sqrt{\frac{Q^2}{gD^3W^3}}$$

where Q = discharge, g = acceleration due to gravity, D = mean depth, and W = mean width. Lamouroux et al. (1992) suggested that mean Froude number of a river section is the most important variable to estimate the parameters of shear stress frequency distributions.

Several solute tracer experiments were carried out in each stream to examine reach transport properties. The tracer was sodium chloride; concentration profiles were measured using pHOX model 52E conductivity meters, each with a Technolog Newlog data logger. The experimental stretches were 50 m long for the smaller streams (Lone Oak and Old Lodge) and 100 m for the larger ones. Tracer mass ranged from 300 g to 6 kg, depending on stream size and discharge. Broadstone Stream is normally highly retentive and slow flowing, which causes difficulties with the solute tracer technique, so the 200-m experimental reach was subdivided into a series of eight 25-m subreaches.

The aggregated dead zone model (ADZ) (Beer and Young 1983; Young and Wallis 1986; Wallis et al. 1989) summarizes the effect of all dispersive processes and dead zones in any given length of stream channel. It employs an empirical, curve-fitting procedure based on time-series analysis and models river

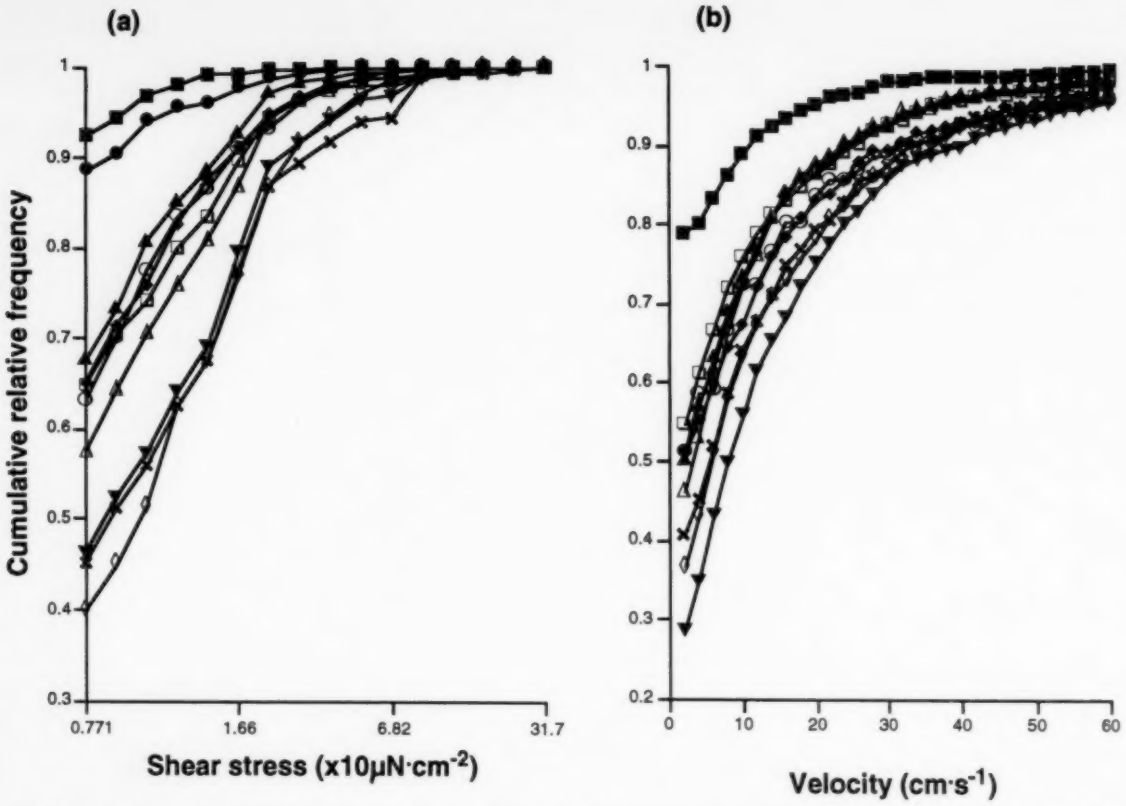


FIG. 4. Cumulative relative frequency of (a) shear stress and (b) velocity in Broadstone Stream at 10 different discharges. See Table 2 for dates, symbols, discharges, Froude numbers, and N values.

discharge as two fractions, one actively flowing and one non-flowing or "dead". The tracer experiment data were analyzed using the ADZ-Analysis computer program (ADZ-Analysis 1991). Using time-series analysis of the solute concentration data (Fig. 3a), the program identifies statistically the appropriate model (Fig. 3b). The coefficient of determination (R_T^2) defines how well the model explains the data; it approaches unity if the fit is very good. The error variance norm (YIC) is a measure of the precision with which the model parameters are estimated; a well-defined model is indicated by a small value (a "large" negative value) as in Fig. 3b. A plot of residual errors between the model and measured data (Fig. 3c) shows a random scatter, further indicating a good fit. Where a model describes the data well, the program provides estimates of some physically meaningful parameters describing the reach characteristics. The parameter most pertinent to the concept of in-stream flow refugia is the dispersive fraction, Df, a measure of the proportion of the total reach volume occupied by dead zones. Because the ADZ of Broadstone Stream was analyzed as a series of eight 25-m subreaches, the dispersive fraction of the entire reach was calculated as

$$\mathrm{Df} = \sum_{i=1}^{i=8} \mathrm{Ve}_i \Big/ \sum_{i=1}^{i=8} V_i$$

where Ve is the dead zone volume and V is the total volume of water in each reach i. Preliminary trials indicated that this procedure was valid.

Results

Flow Surveys

Intensive study: Broadstone Stream

The cumulative relative frequency of shear stresses and near-bed velocities in Broadstone Stream changed with discharge and Froude number (Fig. 4; Table 2). As discharge and Froude number increased, the relative frequency of areas of stream bed with high shear stress and velocity increased also. Note, however, that even at high discharge, a large percentage of the spots have low flows.

The shape of the bottom shear stress frequency distribution in Broadstone Stream changed with discharge, from a unimodal skewed distribution at low discharge to a bimodal distribution at high discharge (Fig. 5a). In addition to these temporal changes in flow conditions, we can make comparisons among separate 25-m subreaches of Broadstone Stream, measured on a single day, at similar discharge, but varying in channel morphology and Froude number (Fig. 5b). Variations in the frequency distribution of shear stress and velocity were again observed. Reaches dominated by glides and pools had the lowest Froude number and unimodal skewed distributions of shear stress. As riffles became more prominent features of the reach, Froude number

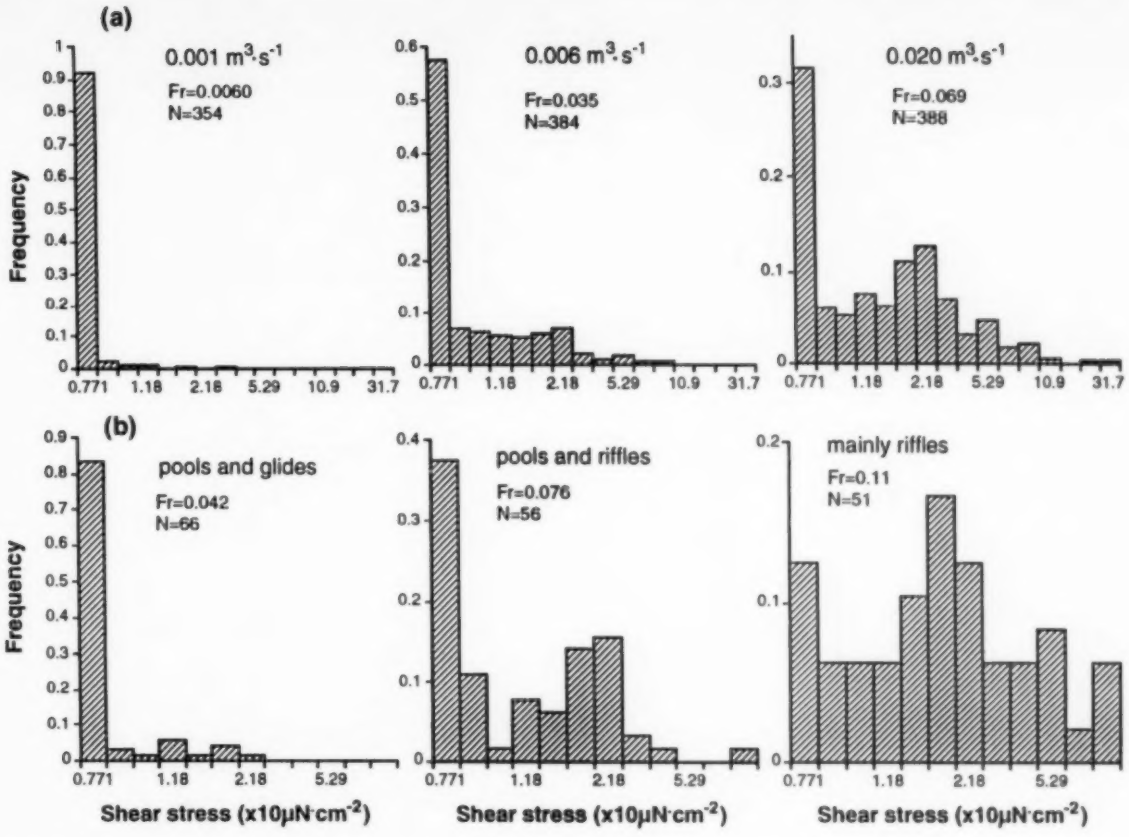


FIG. 5. Frequency distributions of bottom shear stress in Broadstone Stream (a) at three different discharges and (b) at the same discharge (0.020 $m^3 \cdot s^{-1}$) in three 25-m subreaches that differ in channel morphology. Note that y-axis scales differ.

increased, the shear stress distribution became typically bimodal and eventually approached a unimodal bell shape. Note that pattern changes were also observed in the shape of near-bed velocity frequency distributions but, for brevity, only shear stress patterns are presented for all streams.

Hydraulic conditions were measured 10 times at 600 fixed spots and, from this very large data set, a subset of 150 spots was extracted and each spot classified as one of three patch types (Fig. 6): "Slow" patches always had low shear stress and velocity, regardless of discharge. "Fast" patches always had high hydraulic stress, although shear stress may be correlated with discharge. In "variable" patches, shear stress and velocity were low or undetectable at low discharge, not unlike "slow" patches, but increased with discharge and became more similar to "fast" patches. Many spots were unclassified owing to a variety of problems. For example, the water was often very shallow in some spots and, hence, there were too few shear stress and velocity readings for classification. Only the best 50 spots of each type were chosen for illustration.

Comparison streams

The frequency distributions of bottom shear stress at different discharges in the Welsh, Scottish, and remaining Ashdown Forest streams showed an increase in the frequency occurrence of spots with high shear stress and a decrease in the occurrence of low stress, as discharge and Froude number increased. These data, together with those from Broadstone Stream, suggest that there are three patterns of shear stress profiles in these streams: At base flow, Type I streams (Fig. 7a) display a unimodal skewed distribution with low hydraulic stress at a high proportion of the spots and, at high discharge, shift to a bimodal pattern with a greater proportion of high-stress spots, but still many low-stress areas (Broadstone Stream and Old Lodge). At base flow, Type II streams (Fig. 7b) have the same unimodal pattern as Type I streams, but at high discharge shift toward a unimodal bell-shaped pattern with high shear stress in the majority of spots and relatively few or none with low stress (Green Burn, Lone Oak, and Maresfield). Type III streams (Fig. 7c) have base-flow bimodal distributions that shift toward unimodal bell-shaped distributions with few or no low-stress areas at high discharge (Dargall Lane, Afon Hafren, Afon Gwy, and Marsh Green). These patterns suggest that in-stream flow refugia, places that maintain low hydraulic stresses during high discharge, are more abundant in Type I than in Type II or III streams. Type II and III streams have similar conditions at high discharge, but differ in their hydraulic stress patterns at low discharge. At similar Froude number, different streams may have quite different shear stress profiles (cf. Fig. 5b pools and glides versus Fig. 7c base flow; Fig. 7a moderate versus Fig. 7c base flow), indicating that profile shape is not simply dependent on discharge. Similarly,

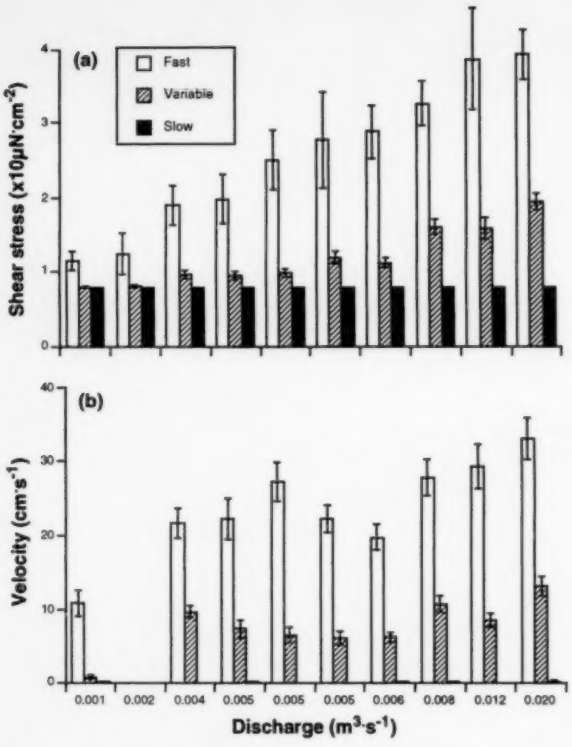


FIG. 6. Mean (±1 SE; $N = 50$) (a) shear stress and (b) velocity in "fast", "variable", and "slow" patches of Broadstone Stream. Kendall's rank correlation coefficients of each data set with discharge: (a) shear stress, fast patches: $\tau = 0.899$, $p < 0.01$; variable; $\tau = 0.764$, $p < 0.01$; slow: $\tau = 0.364$, $p > 0.10$; (b) velocity, fast: $\tau = 0.704$, $p < 0.01$; variable: $\tau = 0.817$, $p < 0.01$; slow: $\tau = 0.309$, $p > 0.10$. Velocity data were not available at 0.002 $m^3 \cdot s^{-1}$ discharge. See Table 2 for dates.

stream type does not simply reflect stream size (see Table 1).

We are confident that the flow surveys were carried out over a sufficiently large discharge range and that the distributions observed at "high discharge" in these streams are representative of conditions under "maximum discharge" even though surveys were not possible under maximum conditions. On at least two occasions, the highest discharge for Dargall Lane (0.543 $m^3 \cdot s^{-1}$) and Afon Hafren (≈0.521 $m^3 \cdot s^{-1}$), the forces of flow were so great that it was nearly impossible and indeed unsafe to conduct the survey. Once the unimodal bell-shaped pattern characteristic of high discharge in Type II and III streams has been reached, any further increase in discharge is likely to shift the profile further to the right, but the profile shape is not expected to change much.

ADZ Analysis

Several solute tracer experiments were carried out on each stream, and all the streams were adequately described by first-order ADZ models with significantly good fits to the data (Table 3). Models with unacceptable R_T^2 and YIC values owing to methodological problems have been omitted. Dispersive fraction, Df, the proportion of the total reach volume occupied by dead zones, did not vary systematically with discharge in any stream, as illustrated for two streams in Fig. 8. Channel morphology and flow patterns are not constant in natural streams and this probably accounts for some of the scatter in Fig. 8.

The dispersive fraction of each stream appears to reflect the prominence of potential in-stream flow refugia in each stream. As defined by shear stress distribution patterns (Fig. 7), Type I streams have the highest Df and have a higher percentage of patches with hydraulic stress at high discharge than either Type II or III streams (Fig. 9). Type III streams may have a slightly higher Df than Type II streams, but it is not clear if this indicates a greater refuge potential or simply more heterogeneous flow patterns. Dispersive fraction does not simply reflect stream size (see Table 1).

Discussion

We examined flow habitat features of streams on a scale relevant to population and community phenomena of benthic macroinvertebrates. Measuring hydraulic forces on the stream bed of natural channels is extremely difficult (Nowell and Jumars 1984; Davis and Barmuta 1989; Statzner and Müller 1989) and, not surprisingly, seldom attempted by ecologists. The methods used in this study are not without their limitations, but were effective for our objectives. Repeat measurements of near-bed velocities and bottom shear stress were correlated and fit the expected relationship reasonably well (Gordon et al. 1992). The techniques used are insensitive to the horizontal direction of flow. This is important because flow patterns in natural channels are extremely heterogeneous and, although the net direction of flow is clear, there are many small-scale variations (Petit 1987). Flow close to the bottom also has a complex three-dimensional structure and the FST hemispheres used to estimate shear stress integrate much of this complexity. Shear stress is perhaps the most meaningful measure of flow as far as benthic organisms are concerned because it is the shearing force of water rather than velocity per se that is likely to erode organisms. Hence, most subsequent discussion will focus on bottom shear stress.

Surveys of hydraulic conditions on the bed of Broadstone Stream over a range of discharges showed that average flow forces increased with stream stage, but that potential low-stress "refugia" were retained at all flows. Using the energy line slope to calculate and map shear stresses in a Belgian river, Petit (1987) also identified discrete areas of river bed with similar patterns of changing shear stress with discharge. Our slow patches satisfy the physical criteria of in-stream flow refugia: they are places that maintain low hydraulic forces throughout the discharge hydrograph. It is not particularly surprising that fast, variable, and slow patches exist in streams, but there is little empirical evidence of these phenomena, and this is essential for future studies on the use of in-stream flow refugia by benthic invertebrates.

Satisfying the physical criteria for in-stream flow refugia is a necessary but not a sufficient condition to demonstrate their biological importance. Until the extent to which animals use these places is determined, at best we can only discuss "potential" refugia or the refuge potential of a stream. High flows are expected to erode animals from fast, and especially from variable, patches on the initial or rising limb of the hydrograph (Borchardt and Statzner 1990). Animals already in slow patches at the onset of the event will not be dislodged, and those entrained in the flow from elsewhere will tend to be deposited in such dead zones. Similarly, Petit (1987) suggested that the greatest erosion

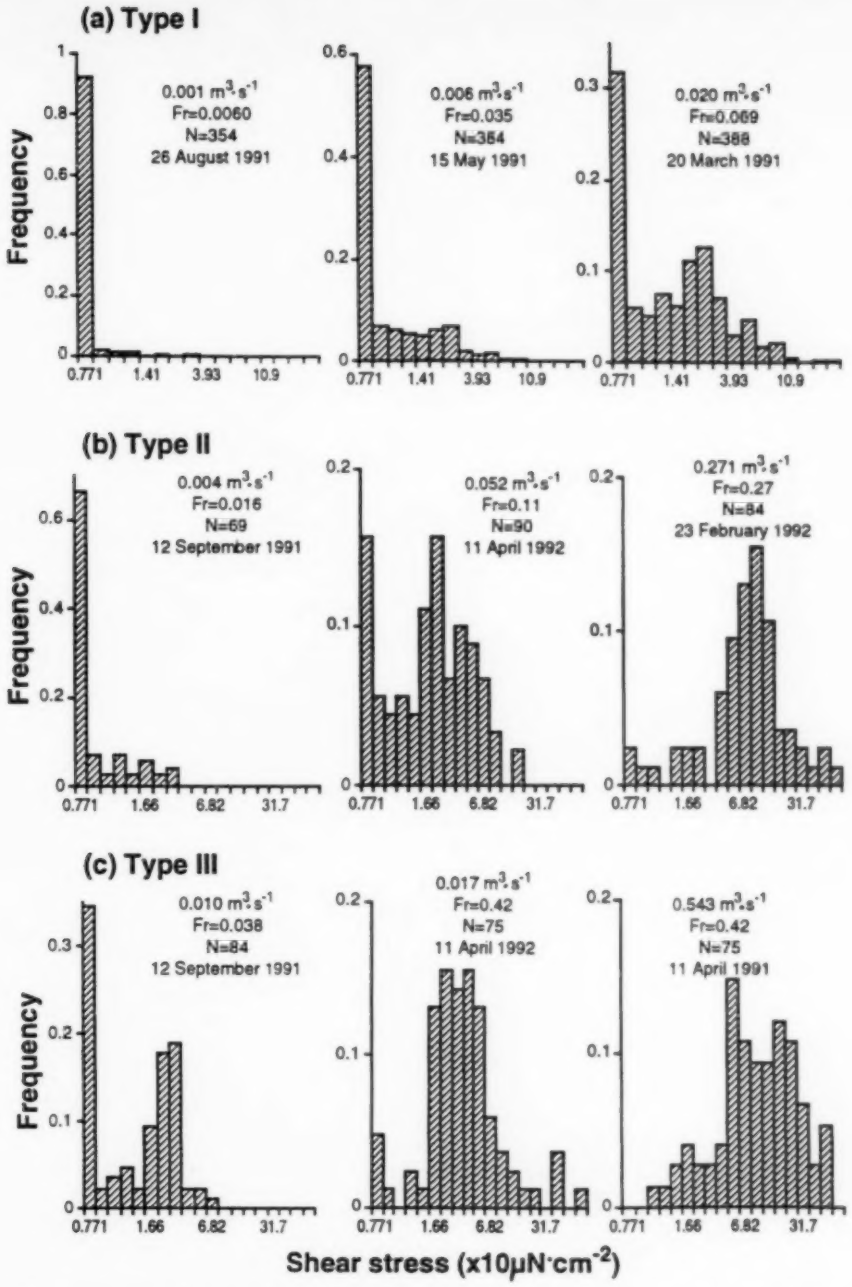


FIG. 7. Frequency distributions of shear stress in three streams representative of Type I (Broadstone Stream), II (Green Burn), and III (Dargall Lane) flow patterns at base flow (lower flows are rarely recorded) and moderate and high discharge.

of pebbles occurs in patches that show the greatest change in shear stress with discharge, and eroded pebbles are deposited in places with relatively weak and constant shear stress. The abundant slow patches in Broadstone Stream are thus places where animals could take refuge from extreme hydraulic forces. Disturbances from high discharge can limit the importance of biotic interactions in controlling community structure (Hemphill and Cooper 1983; Dudley et al. 1990; Lancaster 1990; Peckarsky et al. 1990; Hemphill 1991), so in-stream flow refugia may be particularly important to the Broadstone Stream community, where competition and predation are intense.

Flow surveys revealed distinct shear stress frequency distributions, similar to those recorded by others (Statzner and Müller 1989; Statzner et al. 1990; Lamouroux et al. 1992). The shifts

Table 3. Summary of successful solute tracer experiments showing date, discharge, and statistics (see text for explanation of symbols). Because ADZ volumes for Broadstone Stream were estimated as the sum of a series of 25-m subreaches, statistics are not appropriate here. See text for explanation.

Stream	Date	Discharge ($m^3 \cdot s^{-1}$)	YIC	R_T^2
Broadstone Stream	1 December 1991	0.004		
	5 December 1991	0.003		
	8 December 1991	0.002		
	3 March 1992	0.003		
	12 March 1992	0.004		
	26 March 1992	0.006		
	29 March 1992	0.009		
	11 June 1992	0.001		
Dargall Lane	23 February 1992	0.220	−14.01	0.994
	11 April 1992	0.017	−14.86	0.995
	18 June 1992	0.001	−14.32	0.991
Green Burn	23 February 1992	0.271	−12.49	0.992
	11 April 1992	0.052	−17.29	0.999
	18 June 1992	0.001	−14.18	0.995
Afon Hafren	21 February 1992	0.127	−14.53	0.994
	16 April 1992	0.247	−13.82	0.990
	6 June 1992	0.044	−14.99	0.996
Afon Gwy	22 February 1992	0.134	−13.87	0.989
	16 April 1992	0.211	−13.52	0.987
	17 June 1992	0.050	−14.35	0.992
Lone Oak	22 January 1992	0.013	−13.00	0.982
	15 February 1992	0.027	−13.82	0.991
	11 March 1992	0.010	−14.37	0.996
	19 March 1992	0.005	−15.53	0.997
	27 March 1992	0.011	−15.92	0.996
	25 April 1992	0.023	−15.38	0.995
	28 April 1992	0.180	−15.89	0.997
	12 June 1992	0.003	−16.83	0.997
Old Lodge	27 March 1992	0.026	−14.08	0.988
	25 April 1992	0.048	−15.90	0.997
	28 April 1992	0.283	−12.59	0.980
	23 June 1992	0.010	−12.80	0.971
Maresfield	25 January 1992	0.039	−12.89	0.985
	26 January 1992	0.041	−13.41	0.989
	11 March 1992	0.049	−11.93	0.970
	19 March 1992	0.042	−13.73	0.988
	27 March 1992	0.079	−13.09	0.982
	12 June 1992	0.024	−14.34	0.992

between distribution patterns with changes in discharge and channel morphology are being examined (Lamouroux et al. 1992), but are not fully understood. Three types of stream were identified based on how patterns of hydraulic stress and potential refuge availability varied with discharge.

These stream types probably do not represent discrete patterns in hydraulic stress with changing discharge, but are more likely to be points in a complex array of flow patterns. Poff and Ward (1989) identified nine categories of rivers and streams based on daily discharge patterns over many years. Our streams are all perennial and cover perhaps only three or four categories in their classification scheme (snow and rain, winter rain, mesic groundwater, and perennial runoff); clearly, other hydraulic patterns are possible. Similarly, channel morphology varies among streams and this also influences hydraulic patterns. Shear stress distribution patterns of subreaches of Broadstone Stream measured on the same day, at the same discharge, differed with the morphology of each subreach. Lamouroux et al. (1992) found that streams with the same discharge range can have different shear stress patterns, and streams with different discharge ranges can have similar patterns. Clearly, discharge alone is insufficient to predict the physical properties of bottom flow, and the relationship with morphology is complex.

Predictions can be made about the kind of species assemblage present in a particular stream, its persistence, resilience, and the strength of species interactions based on stream type. We need to distinguish two kinds of potential effects of these differences in channel hydraulics on the biota. Firstly, the species assemblage may reflect the hydraulic habitat at base flow, which is the most commonly occurring stage in most streams (Newbury 1984), including those we studied. Secondly, the community and its persistence could be determined by the provision of refugia from high shear stress at peak flows (a reflection of heterogeneity in the near-bed fluid habitat). This is a distinction between

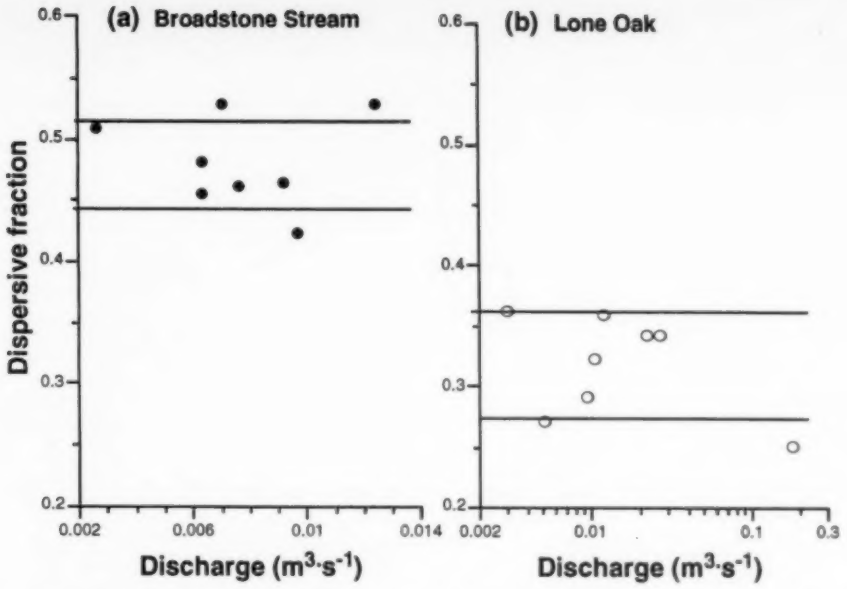


FIG. 8. Dispersive fraction of (a) Broadstone Stream and (b) Lone Oak measured over a range of discharge. Horizontal lines indicate standard deviations about the mean.

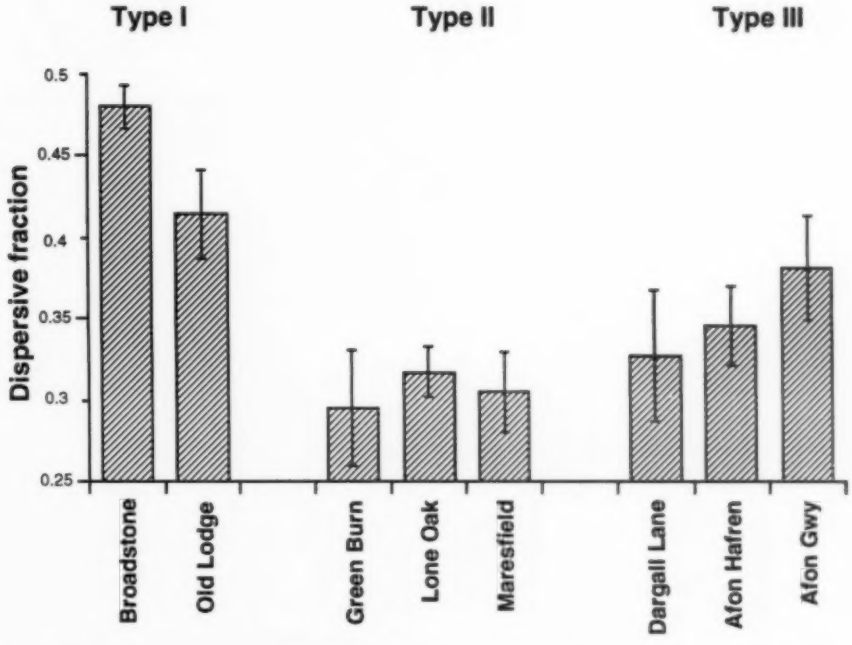


FIG. 9. Mean dispersive fraction (±1 SE) of eight streams arranged according to type, at the various discharges indicated in Table 3. Note that solute tracer experiments were not possible in the ninth stream, Marsh Green, owing to access difficulties.

adjustment to long-term conditions or to structuring by episodic disturbance, and these processes may be difficult to disentangle. For instance, one might predict that Type I streams are dominated by species that prefer slow-flowing water because low shear stress conditions predominate throughout the year. Even at high discharge, there are still many areas of low stress and hence abundant in-stream flow refugia in which animals can avoid physical disturbances. Rheophilous species are likely to be

uncommon because areas of fast-flowing water are scarce except during rare spates. In contrast, rheophilous species may be common in Type III streams, which have many areas of fast-flowing water even at base flow. Although slow-flowing areas may exist at base flow, species restricted to slow water may be rare because there are few areas of low stress and hence few in-stream flow refugia, even at moderate discharge. There is some evidence that among-channel variations in flow pattern are in fact reflected by communities of macroinvertebrates (e.g., Morgan et al. 1991) and lotic algae (e.g., Peterson and Stevenson 1992). There may also be intraspecific, within-population variations among streams that are related to flow characteristics. For example, Robinson et al. (1992) observed greater genetic variability in populations of a stonefly, *Hesperoperla pacifica*, and a mayfly, *Baetis tricaudatus*, in a stream with high flow variability (i.e., snow melt, seasonally cyclic flow regime) compared with more stable flows (mesic ground water, seasonally constant).

Application of transient storage models and hydraulic dead zones is a novel approach to examining the refuge potential and transport of stream reaches with respect to benthic invertebrate communities. Reynolds (1988) recognized the importance of dead zones in maintaining lotic populations of planktonic algae, and Reynolds et al. (1991) showed that the spatial distribution of these organisms does track the velocity structure of river channels. For the first time, we have used the ADZ model (Beer and Young 1983; Young and Wallis 1986; Wallis et al. 1989) to quantify the availability of in-stream flow refugia for macroinvertebrates in streams. All our stream reaches were described adequately by first-order ADZ models, as has been observed for most other systems (Wallis et al. 1989), and hence it was possible to estimate physically meaningful parameters describing the reach characteristics. The dispersive fraction, the proportion of the total reach volume occupied by hydraulic dead zones, did not vary with discharge in any stream, a similar result to that reported by Wallis et al. (1989). This parameter is perhaps most pertinent to the concept of in-stream flow refugia: a low dispersive fraction is likely to be associated with stream reaches that have (1) few flow refugia where animals can avoid erosion from the substrate by extreme hydraulic forces and (2) water column flow structures likely to transport drifting invertebrates out of the reach. The dispersive fraction of Type I streams was greater than of either Type II or III, in parallel with patterns of their refuge potential. Differences in the refuge availability in Type II and III streams are less clear and require further investigation.

Our analysis of the complex physical habitat of running-water habitats is consistent with the hypotheses that in-stream flow refugia are prominent in some streams and that the prominence of flow refugia varies among streams. There are, however, several questions that need to be answered, and are currently being addressed, regarding the importance of these potential refugia to the benthic invertebrate community. Do animals actually use these refugia? Are the hydraulic transport properties of streams reflected by the transport or drift patterns of animals? Are differences in refuge potential and transport properties among streams related to differences in patterns of community structure and persistence?

Finally, we have emphasized the potential importance of the fluid habitat as a factor intervening directly in population dynamics (by providing refugia from spates). It is important to recall, however, that there are also "indirect" effects through a variety of interacting ecological processes. For instance, flow forces and transport processes determine the retention of both coarse and fine organic particles, which affect animal populations through their food supply (Richardson 1991; Dobson and Hildrew 1992). Nutrient spiralling and the processing of dissolved organics must also be affected by the hydraulic habitat (Elwood et al. 1983; Meyer et al. 1988). Characterizing such key aspects of the physical habitat of streams will prove central to increasing understanding at all levels in lotic ecology.

Acknowledgements

This project would not have been possible without the field assistance of many people, often in the most diabolical weather, and we are grateful to them all: Stuart Orton, Paul Garner, Anne Robertson, Ronni Edmonds-Brown, David Dobson, and Julie Winterbottom. Bernhard Statzner and Seth Reice commented on earlier versions of this paper. Discharge data were kindly provided by the National Rivers Authority (Southern Region), the Institute of Hydrology, and the Solway River Purification Board. We thank the various land owners for permitting access to the stream sites. This project was funded by a grant from the Natural Environment Research Council.

References

ADZ-Analysis. 1991. ADZ-Analysis program. Centre for Research on Environmental Systems, Lancaster University, Lancaster, England.

Badri, A., J. Giudicelli, and G. Prévot. 1987. Effects of a flood on the benthic invertebrate community in a Mediterranean river, the Rdat (Morocco). Acta Oecol. 8: 481–500.

Beer, T., and P.C. Young. 1983. Longitudinal dispersion in natural streams. J. Environ. Eng. Am. Soc. Civ. Eng. 109: 1047–1067.

Bencala, K.E., and R.A. Walters. 1983. Simulation of solute transport in a mountain pool-and-riffle stream: a transient storage model. Water Resour. Res. 19: 718–724.

Borchardt, D., and B. Statzner. 1990. Ecological impact of urban storm water runoff studied in experimental flumes — population loss by drift and availability of refuge space. Aquat. Sci. 52: 299–314.

Boulton, A.J., C.G. Peterson, N.B. Grimm, and S.G. Fisher. 1992. Stability of an aquatic macroinvertebrate community in a multiyear hydrologic disturbance regime. Ecology 73: 2192–2207.

Carling, P.A. 1992. The nature of the fluid boundary-layer and the selection of parameters for benthic ecology. Freshwater Biol. 28: 273–278.

Davis, J.A., and L.A. Barmuta. 1989. An ecologically useful classification of mean and near-bed flows in streams and rivers. Freshwater Biol. 21: 271–282.

Delucchi, C.M. 1988. Comparison of community structure among streams with different temporal flow regimes. Can. J. Zool. 66: 579–586.

Delucchi, C.M. 1989. Movement patterns of invertebrates in temporary and permanent streams. Oecologia 78: 199–207.

Dobson, M.K., and A.G Hildrew. 1992. A test of resource limitation among shredding detritivores in low order streams in southern England. J. Anim. Ecol. 61: 69–78.

Dole-Olivier, M-J., and P. Marmonier. 1992. Effects of spates on the vertical distribution of the interstitial community. Hydrobiologia 230: 49– 61.

Dudley, T.L., C.M. D'Antonio, and S.D. Cooper. 1990. Mechanisms and consequences of interspecific competition between two stream insects. J. Anim. Ecol. 59: 849–866.

Elwood, J.W., J.D. Newbold, R.V. O'Neill, and W. Van Winkle. 1983. Resource spiraling: an operational paradigm for analyzing lotic systems, p. 3–27. *In* T.D. Fontaine and S.M. Bartell [ed.] Dynamics of lotic ecosystems. Ann Arbor Science Publishers, Ann Arbor, Mich.

Fischer, H.B. 1967. The mechanics of dispersion in natural streams. J. Hydraul. Div. Am. Soc. Civ. Eng. 93: 187–216.

Giberson, D.J., and R.J. Hall. 1988. Seasonal variation in faunal distribution within the sediments of a Canadian Shield stream, with emphasis on responses to spring floods. Can. J. Fish. Aquat. Sci. 45: 1994–2002.

Giller, P.S., N. Sangpraduh, and H. Twomey. 1991. Catastrophic flooding and macroinvertebrate community structure. Verh. Int. Ver. Limnol. 24: 1724–1729.

Gordon, N.D., T.A. McMahon, and B.L. Finlayson. 1992. Stream hydrology: an introduction for ecologists. John Wiley & Sons, Inc., Chichester, England. 526 p.

HEMPHILL, N. 1991. Disturbance and variation in competition between two stream insects. Ecology 72: 864–872.

HEMPHILL, N., AND S.D. COOPER. 1983. The effect of physical disturbance on the relative abundances of two filter-feeding insects in a small stream. Oecologia 58: 378–382.

HILDREW, A.G., AND P.S. GILLER. 1994. Patchiness, species interactions and disturbance in the stream benthos. *In* P.S. Giller, A.G. Hildrew, and D. Raffaelli [ed.] Aquatic ecology: scale pattern and process. Blackwell Scientific Publications, Oxford. (In press)

HILDREW, A.G., AND C.R. TOWNSEND. 1976. The distribution of two predators and their prey in an iron-rich stream. J. Anim. Ecol. 45: 41–57.

HILDREW, A.G., AND C.R. TOWNSEND. 1980. Aggregation, interference and foraging by larvae of *Plectrocnemia conspersa* (Trichoptera: Polycentropodidae). Anim. Behav. 28: 553–560.

HILDREW, A.G., AND C.R. TOWNSEND. 1982. Predators and prey in a patchy environment: a freshwater study. J. Anim. Ecol. 51: 797–815.

LAMOUROUX, N., B. STATZNER, U. FUCHS, F. KOHMANN, AND U. SCHMEDTJE. 1992. An unconventional approach to modelling spatial and temporal variability of local shear stress in stream segments. Water Resour. Res. 28: 3251–3258.

LANCASTER, J. 1990. Predation and drift of lotic macroinvertebrates during colonization. Oecologia 85: 48–56.

LANCASTER, J., A.G. HILDREW, AND C.R. TOWNSEND. 1988. Competition for space by predators in streams: field experiments on a net-spinning caddisfly. Freshwater Biol. 20: 185–193.

LANCASTER, J., A.G. HILDREW, AND C.R. TOWNSEND. 1990. Stream flow and predation effects on the spatial dynamics of benthic invertebrates. Hydrobiologia 203: 177–190.

LANCASTER, J., A.G. HILDREW, AND C.R. TOWNSEND. 1991. Invertebrate predation on patchy and mobile prey in streams. J. Anim. Ecol. 60: 625– 641.

MARMONIER, P., AND M. CREUZÉ DES CHÂTELLIERS. 1991. Effects of spates on interstitial assemblages of the Rhône River. Importance of spatial heterogeneity. Hydrobiologia 210: 243–251.

MEYER, J.L., W.H. MCDOWELL, T.L. BOTT, J.W. ELWOOD, C. ISHIZAKI, J.M. MELACK, B.L. PECKARSKY, B.J. PETERSON, AND P.A. RUBLEE. 1988. Elemental dynamics in streams. J. North Am. Benthol. Soc. 7: 410–432.

MORGAN, R.P., R.E. JACOBSEN, S.B. WEISBERG, L.A. MCDOWELL, AND H.T. WILSON. 1991. Effects of flow alteration on benthic macroinvertebrate communities below the Brighton hydroelectric dam. J. Freshwater Ecol. 6: 419–429.

NEWBURY, R. 1984. Hydrologic determinants of aquatic insect habitats, p. 323–357. *In* V.H. Resh and D.M. Rosenberg [ed.] The ecology of aquatic insects. Praeger, New York, N.Y.

NIEMI, G.J., P. DEVORE, N. DETENBECK, D. TAYLOR, A. LIMA, J. PASTOR, J.D. YOUNT, AND R.J. NAIMAN. 1990. Overview of case studies on recovery of aquatic systems from disturbance. Environ. Manage. 14: 571–587.

NOWELL, A.R.M., AND P.A. JUMARS. 1984. Flow environments of aquatic benthos. Annu. Rev. Ecol. Syst. 15: 303–328.

PALMER, M.A., A.E. BELY, AND K.A. BERG. 1992. Responses of invertebrates to lotic disturbances: a test of the hyporheic refuge hypothesis. Oecologia 89: 182–194.

PECKARSKY, B.L., S.C. HORN, AND B. STATZNER. 1990. Stonefly predation along a hydraulic gradient: a field test of the harsh–benign hypothesis. Freshwater Biol. 24: 181–191.

PETERSON, C.G., AND R.J. STEVENSON. 1992. Resistance and resilience of lotic algal communities: importance of disturbance timing and current. Ecology 73: 1445–1461.

PETIT, F. 1987. The relationship between shear stress and the shaping of the bed of a pebble-loaded river in La Rulles – Ardenne. Catena 14: 453– 468.

POFF, N.L., AND J.V. WARD. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. Can. J. Fish. Aquat. Sci. 46: 1805–1818.

POFF, N.L., AND J.V. WARD. 1990. Physical habitat template of lotic systems: recovery in the context of historical pattern of spatiotemporal heterogeneity. Environ. Manage. 14: 629–645.

POOLE, W.C., AND K.W. STEWART. 1976. The vertical distribution of macrobenthos within the substratum of the Bazos River, Texas. Hydrobiologia 50: 151–160.

REYNOLDS, C.S. 1988. Potamoplankton: paradigms, paradoxes and prognoses, p. 285–311. *In* F.E. Round [ed.] Algae and the aquatic environment: contributions in the honour of J.W.G. Lund. Biopress, Bristol.

REYNOLDS, C.S., P.A. CARLING, AND K.J. BEVEN. 1991. Flow in river channels: new insights into hydraulic retention. Arch. Hydrobiol. 121: 171–179.

RICHARDSON, J.S. 1991. Seasonal food limitation of detritivores in a montane stream: an experimental test. Ecology 72: 873–887.

ROBINSON, C.T., L.M. REED, AND G.W. MINSHALL. 1992. Influence of flow regime on life history, production, and genetic structure of *Baetis tricaudatus* (Ephemeroptera) and *Hesperoperla pacifica* (Plecoptera). J. North Am. Benthol. Soc. 11: 278–289.

SCHLOSSER, I.J. 1991. Stream fish ecology: a landscape perspective. BioScience 41: 704–712.

SEDELL, J.R., G.H. REEVES, F.R. HAUER, J.A. STANFORD, AND C.P. HAWKINS. 1990. Role of refugia in recovery from disturbances: modern fragmented and disconnected river systems. Environ. Manage. 14: 711–724.

SOUTHWOOD, T.R.E. 1977. Habitat, the templet for ecological strategies? J. Anim. Ecol. 46: 337–365.

SOUTHWOOD, T.R.E. 1988. Tactics, strategies and templets. Oikos 52: 3–18.

STANFORD, J.A., AND A.R. GAUFIN. 1974. Hyporheic communities of two Montana rivers. Science (Wash., D.C.) 185: 700–702.

STATZNER, B., AND B. HIGLER. 1986. Stream hydraulics as a major determinant of benthic invertebrate zonation patterns. Freshwater Biol. 16: 127–139.

STATZNER, B., F. KOHMANN, AND A.G. HILDREW. 1991. Calibration of FST-hemispheres against bottom shear stress in a laboratory flume. Freshwater Biol. 26: 227–231.

STATZNER, B., F. KOHMANN, AND U. SCHMEDTJE. 1990. Eine methode zur okologischen Bewertung van Restabflussen in Ausleitungsstrecken. Wasserwirtschaft 80: 248–254.

STATZNER, B., AND R. MÜLLER. 1989. Standard hemispheres as indicators of flow characteristics in lotic benthic research. Freshwater Biol. 21: 445– 460.

TOWNSEND, C.R., AND A.G. HILDREW. 1976. Field experiments on the drifting, colonization, and continuous redistribution of stream benthos. J. Anim. Ecol. 45: 759–772.

TOWNSEND, C.R., AND A.G. HILDREW. 1994. Species traits in relation to a habitat templet for river systems. Freshwater Biol. (In press)

TOWNSEND, C.R., A.G. HILDREW, AND J. FRANCIS. 1983. Community structure in some southern English streams: the influence of physiochemical factors. Freshwater Biol. 13: 521–544.

TOWNSEND, C.R., A.G. HILDREW, AND K. SCHOFIELD. 1987. Persistence of stream invertebrate communities in relation to environmental variability. J. Anim. Ecol. 56: 597–614.

WALLIS, S.G., P.C. YOUNG, AND K.J. BEVEN. 1989. Experimental investigation of the aggregated dead zone model for longitudinal solute transport in stream channels. Proc. Inst. Civ. Eng. 87: 1–22.

WILLIAMS, D.D. 1984. The hyporheic zone as a habitat for aquatic insects and associated arthropods, p. 430–455. *In* V.H. Resh and D.H. Rosenberg [ed.] The ecology of aquatic insects. Praeger, New York, N.Y.

WILLIAMS, D.D., AND H.B.N. HYNES. 1974. The occurrence of benthos deep in the substratum of a stream. Freshwater Biol. 4: 233–256.

YOUNG, P.C., AND S.G. WALLIS. 1986. The aggregated dead zone model for dispersion in rivers, p. 421–433. *In* Proceedings of the Conference on Water Quality Modelling in the Inland Natural Environment. B.H.R.A., Cranfield, England.

Hormonal Control of Hepatic Glycogen Metabolism in Food-deprived, Continuously Swimming Coho Salmon (*Oncorhynchus kisutch*)

M.M. Vijayan[1]

Department of Biology, University of Ottawa, Ottawa, ON K1N 6N5, Canada

A.G. Maule[2]

Department of Fisheries and Wildlife, Oregon Cooperative Fishery Research Unit, Oregon State University, Corvallis, OR 97331-3803, USA

C.B. Schreck

Department of Fisheries and Wildlife, Oregon Cooperative Fishery Research Unit, Oregon State University, Corvallis, OR 97331-3803, USA

and T.W. Moon

Department of Biology, University of Ottawa, Ottawa, ON K1N 6N5, Canada

Vijayan, M.M., A.G. Maule, C.B. Schreck, and T.W. Moon. 1993. Hormonal control of hepatic glycogen metabolism in food-deprived, continuously swimming coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 50: 1676–1682.

The plasma cortisol concentration and liver cytosolic glucocorticoid receptor activities of continuously swimming, food-deprived coho salmon (*Oncorhynchus kisutch*) did not differ from those of resting, fed fish. Plasma glucose concentration was significantly higher in the exercising, starved fish, but there were no significant differences in either hepatic glycogen concentration or hepatic activities of glycogen phosphorylase, glycogen synthase, pyruvate kinase, or lactate dehydrogenase between the two groups. Total glucose production by hepatocytes did not differ significantly between the two groups; glycogen breakdown accounted for all the glucose produced in the resting, fed fish whereas it explained only 59% of the glucose production in the exercised animals. Epinephrine and glucagon stimulation of glucose production by hepatocytes was decreased in the exercised fish without significantly affecting hepatocyte glycogen breakdown in either group. Insulin prevented glycogen breakdown and enhanced glycogen deposition in exercised fish. The results indicate that food-deprived, continuously swimming coho salmon conserve glycogen by decreasing the responsiveness of hepatocytes to catabolic hormones and by increasing the responsiveness to insulin (anabolic hormone).

Les concentrations de cortisol plasmatique et l'activité des récepteurs glucocorticoïdes de l'hyaloplasme hépatique de saumons cohos (*Oncorhynchus kisutch*) qu'on fait jeûner et qu'on force à nager sans interruption ne diffèrent pas des concentrations et de l'activité observées chez des congénères nourris et au repos. La concentration de glucose plasmatique est considérablement plus forte chez le poisson mis au jeûne et à l'exercice, mais il n'y a aucune différence notable, soit dans la concentration de glycogène hépatique, soit dans l'activité hépatique de la glycogène-phosphorylase, de la glycogène-synthétase, de la pyruvate-kinase ou de la lactate-déshydrogénase entre les deux groupes de poissons. La production totale de glucose chez les hépatocytes ne différait pas beaucoup entre les deux groupes; la dégradation du glycogène comptait pour toute la production de glucose chez les poissons nourris et au repos, tandis qu'elle ne comptait que pour 59% de la production chez l'animal actif. La stimulation de la production de glucose par les hépatocytes grâce à l'épinéphrine et au glucagon a diminué chez le poisson actif, sans influer considérablement sur la dégradation du glycogène par les hépatocytes chez aucun groupe de poissons. L'insuline a empêché la dégradation du glycogène et amélioré son stockage chez le poisson en activité. Les résultats montrent qu'un coho nageant sans interruption et privé de nourriture conserve son glycogène en diminuant la réaction des hépatocytes aux hormones du catabolisme et en accroissant leur réaction à l'insuline (hormone anabolisante).

Received October 23, 1992
Accepted February 26, 1993
(JB669)

Reçu le 23 octobre 1992
Accepté le 26 février 1993

Hepatic glycogen stores are utilized for immediate energy requirements by fish in stressful situations (see Vijayan and Moon 1992). Several hormones, including catecholamines, pancreatic hormones, and glucocorticoids, influence hepatic glycogenolysis (Mommsen et al. 1988; Wright et al. 1989; Sheridan and Mommsen 1991; Vijayan and

[1]Author to whom all correspondence should be addressed. Present address: EVS Environment Consultants, 195 Pemberton Avenue, North Vancouver, BC V7P 2R4, Canada.

[2]Present address: U.S. Fish and Wildlife Service, National Fishery Research Station, Columbia River Field Station, Cook, WA 98605, USA.

Leatherland 1992) and thus are potentially involved in stress-induced hepatic glycogen mobilization (Vijayan and Moon 1992). It is important therefore that fish maintain hepatic glycogen stores to cope with the increased energy demand associated with stress (Vijayan and Moon 1992).

Fish maintain liver glycogen content by mobilizing other energy stores such as lipids and proteins. Not surprisingly, the prior nutritional state of the animal plays an important role in liver glycogen metabolism (Sheridan and Mommsen 1991; Vijayan and Moon 1992). Recently, Vijayan and Moon (1992) showed that food-deprived rainbow trout (*Oncorhynchus mykiss*) were in a negative energy balance and hence unable to replenish their hepatic glycogen after acute stress, resulting in glycogen depletion.

In natural situations, certain fish species including salmonids do not feed during migration (Beamish 1964). The energetic costs during migration are high, and this energy drain is accentuated because the fish do not feed. Fish have developed strategies for coping with periods of food deprivation whereby liver glycogen content is either conserved or partially conserved at the expense of lipids and proteins (see Sheridan and Mommsen 1991 for references). However, little is known about the strategies adopted by fish to maintain liver glycogen content in situations of chronic energy expenditure (such as migration).

French et al. (1981) showed that the combination of continuous swimming and fasting in rainbow trout resulted in a two-fold increase in gluconeogenesis from amino acids with a concomitant increase in the activity of liver phosphoenolpyruvate carboxykinase. Similar results were obtained for migrating salmon (Mommsen et al. 1980; French et al. 1983), suggesting that amino acids are mobilized to provide glucose, thereby conserving liver glycogen. Coho salmon deprived of food for 3 wk partially conserved liver glycogen and plasma glucose while mobilizing lipids (Sheridan and Mommsen 1991).

Cortisol has been implicated as a metabolic hormone in fish, increasing gluconeogenesis from amino acids and glycerol to cope with enhanced energy demand during stress (Foster and Moon 1986; Sheridan 1986; Vijayan et al. 1991). Cortisol also has a direct and/or indirect effect on hepatic glycogen metabolism (Vijayan and Leatherland 1992; Vijayan and Moon 1992). Because other glucoregulatory hormones including catecholamines (Mommsen et al. 1988; Wright et al. 1989; Brighenti et al. 1991) and pancreatic hormones (Plisetskaya 1990; Mommsen and Plisetskaya 1991) affect glycogen metabolism, it is possible that cortisol might exert a permissive and/or interactive effect with these other hormones on hepatic glycogen metabolism. In support of this statement, a recent study showed that cortisol treatment modified the hepatocyte response to epinephrine stimulation of glycogen metabolism in the sea raven (*Hemitripterus americanus*) (Vijayan et al. 1993). However, brook trout (*Salvelinus fontinalis*) that received slow-release cortisol implants showed altered activity of some hepatic enzymes, even though circulating cortisol levels did not differ from controls beyond 24 h after implantation (Vijayan et al. 1991). Similarly, immune dysfunction in chinook salmon (*Oncorhynchus tshawytscha*) occurred 7 d after an acute stress and 6 d after cortisol levels returned to control values (Maule et al. 1989). Also, the number of glucocorticoid receptors (GR) in coho salmon (*Oncorhynchus kisutch*) gill cytosol was reduced significantly at 7 d after a single meal containing exogenous cortisol (Maule and Schreck 1991). Thus, it appears that even in situations where plasma cortisol concentrations return to control levels within a relatively short time, the effects of transient increases in that hormone may last over a longer term and may be reflected in cortisol receptors.

The purpose of the present study was to test the hypothesis that hepatocyte sensitivity to hormonal stimulation of glycogenolysis is lowered to conserve glycogen in food-deprived, continuously swimming coho salmon. The study also examined target tissue sensitivity to cortisol in these fish by measuring GR numbers and affinity in liver cytosol.

Materials and Methods

Animals

Juvenile coho salmon obtained from Eagle Creek National Fish Hatchery were acclimated to conditions at the Oregon State University Smith Farm Experimental Hatchery, Corvallis, Oreg., for many months. The fish were kept in circular tanks (about 1700 L) continuously supplied with well water at 11– 12°C and maintained under natural photoperiod. The fish were fed Oregon Moist Pellets at 1–2% of body weight daily.

Experimental Protocol

All experiments were carried out between mid-August and mid-September. Groups of 15 fish (average body weight 200 g) were randomly assigned to one of four experimental tanks (1 m in diameter). Two tanks were randomly chosen for the continuously swimming fish. This group was chosen to mimick the conditions experienced by migrating fish, so the fish were not fed. The experimental protocol was not designed to separate the effects of swimming and food deprivation. The tanks for continuous swimming had the water level lowered to 30 cm to give an average current speed of approximately 1 body length (bl) (22 cm) per second. The other two tanks (water depth 90 cm; velocity 1 $cm \cdot s^{-1}$) held the resting fish that were fed as those in the stock tanks. The lowering of water levels in the tank containing the swimming fish could have resulted in densities different from those in the tanks containing the resting fish. However, the densities of fish in all the tanks (both resting and swimming) were low and not expected to affect the outcome of the experiment (Vijayan et al. 1990). Feeding was stopped 1 wk prior to sampling the resting fish to discount any effect of food on metabolism. The fish were sampled 3 wk after the start of the experiment.

Experiment 1: Hepatic Enzymes and Glucocorticoid Receptors

Samples were taken in the morning between 09:00 and 11:00. Four fish, randomly selected from each treatment, were quickly netted and anaesthetized in a 1 : 10 000 (w/v) solution of neutralized (with sodium bicarbonate) 3-aminobenzoic acid, ethyl ester (MS222, Sigma Chemical Co., St. Louis, Mo.). Blood was collected into heparinized tubes after severing the caudal peduncle, and the plasma, separated by centrifugation, was stored frozen at −80°C. The mass of each fish was recorded to determine the hepatosomatic index (HSI). The liver was removed quickly, weighed (to determine HSI), and a piece frozen in liquid nitrogen. Livers remained frozen for 2 wk at −80°C until analyzed for glycogen content and enzyme activities. The remaining piece of liver was homogenized immediately for the cortisol receptor assay.

Enzymes

Hepatic enzyme activities were estimated according to Moon et al. (1989). Briefly, frozen liver pieces were sonicated (Kontes cell disrupter) in four volumes of a phosphorylation–dephosphorylation "stopping buffer" (Foster and Moon 1989) and centrifuged for 10 min at 12 000 × *g*. The supernatant was applied to a Sephadex G-25 column (Moon et al. 1989), and the eluate was used for measuring enzyme activities at 10°C using a Beckman DU-65 recording spectrophotometer. Enzyme activity was expressed in units (micromoles per minute) per gram of tissue.

Glycogen phosphorylase (GPase) assay — A two-buffer system was used to distinguish GPase *a* from total (*a* + *b*) GPase. The ratio of GPase activities with (GPase *a*) and without caffeine (total GPase), but in the presence of AMP, represents the percentage of total GPase (*a* + *b*) in the active form (*a*) (% GPase *a*) (Moon et al. 1989).

Glycogen synthase (GSase) assay — A two-buffer system was used to distinguish GSase *a* (20 mM UDPG) from total GSase (5 mM glucose-6-phosphate (G6P) with 1.5 mM UDPG) using the spectrophotometric assay of Passonneau and Rottenberg (1973). Other components of the assay were 100 mM KCl, 10 mM $MgCl_2$, 2 mg dialysed glycogen·mL^{-1}, 5 mM phosphoenolpyruvate (PEP), 0.15 mM NADH, and 10 units of pyruvate kinase — lactate dehydrogenase (PK/LDH) in 50 mM imidazole buffer (pH 7.8).

Pyruvate kinase (PK) assay — PK activity was estimated according to Perry et al. (1988) using a 50 mM imidazole buffer (pH 7.5), 100 mM KCl, 10 mM $MgCl_2$, 2.5 mM ADP, 0.15 mM NADH, and 10 units of PK-free LDH. Activities were determined at 0.1 and 5 mM PEP concentrations and an activity ratio was calculated as the activity at low substrate concentration over that at high substrate concentration. Changes in this ratio are indicative of the state of PK phosphorylation in this species (Mommsen and Suarez 1984; Perry et al. 1988).

Lactate dehydrogenase (LDH) assay — LDH activity was estimated spectrophotometrically with 1 mM pyruvate and 0.15 mM NADH in a 50 mM imidazole buffer (pH 7.8).

Glucocorticoid receptor (GR) assay

GR assays of whole liver cytosol were essentially as has been described for gill (Maule and Schreck 1990). Briefly, individual pieces of liver were processed in ice-cold buffer (10 mM Tris–HCl, 1 mM EDTA, 12 mM monothioglycerol, 20 mM sodium molybdate, and 10% (v/v) glycerol; pH 7.4) and disrupted using a motor-driven glass and teflon homogenizer. After an initial centrifugation (2000 × *g* for 20 min) to remove gross debris, the homogenate was centrifuged at 100 000 × *g* for 60 min in an L8-70 ultracentrifuge with a type 40 rotor (Beckman Instruments, Palo Alto, Calif.). Supernatant was added to a buffer containing 5% (w/v) Norit A charcoal and 0.5% Dextran in a 2:1 ratio and then centrifuged to remove endogenous steroids. Protein content in the sample was assayed with the Bradford reagent (Bradford 1976) using bovine serum albumin as a standard and the protein content was adjusted to 5–8 mg·mL^{-1}. Samples were incubated for total binding in buffer or for non-specific binding (NSB) in a 100-fold excess of radioinert triamcinolone acetonide (TA; 1,4-pregnadien-9-fluoro-11,16,17,21-tetrol-3,20-dione-16,17 acetonide; specific activity 25.9 Ci/mmol (1 Ci = 37 GBq); Amersham, Arlington Heights, Ill.) for 10 min, followed by the addition of sufficient radiolabeled TA in buffer to give 0.5–30.0 nmol hormone·L^{-1} in a final volume of 0.2 mL. After 2 h, unbound hormone was separated from bound hormone by centrifugation (3000 × *g*, 20 min) with 0.5 mL of 2.5% (w/v) Norit A charcoal and 0.25% (w/v) Dextran in buffer (10-min incubation). The radioactivity was determined by adding 5.0 mL of scintillation cocktail (Budget-Solve, Research Products International, Mount Prospect, Ill.) to 0.5 mL of the final supernatant and counted on a model LS 1800 liquid scintillation counter (Beckman Instruments).

Experiment 2: Hepatocyte Preparation and Incubation

Another four fish from each treatment were anaesthetized in neutralized MS-222 as mentioned above and blood collected rapidly by caudal puncture for measuring plasma cortisol, glucose, and lactate concentrations. The hepatic portal vein was then quickly cannulated for liver perfusion and the hepatocytes were prepared by collagenase treatment according to Foster and Moon (1989). Cells were resuspended in a medium containing 110 mM NaCl, 3 mM KCl, 1.25 mM KH_2PO_4, 5 mM $NaHCO_3$, 0.6 mM $MgSO_4$, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, and 1.5% fatty-acid-free bovine serum albumin, and adjusted to pH 7.8. The weight of the cells in the medium ranged from 36 to 87 mg·mL^{-1}.

Hepatocytes were incubated in a 1-mL aliquot either without hormones or with either epinephrine (10^{-5} M), a mixture of bovine and porcine glucagon (10^{-8} M), or bovine insulin (10^{-8} M). Incubations proceeded for 1 h at 10°C and were terminated by the addition of 25 µL of 35% perchloric acid (PCA). A sample was acidified before incubation for estimates of initial glycogen and glucose. Acidified samples were stored frozen at –80°C until analysis.

Plasma and tissue metabolite assays

Plasma cortisol was assayed using a radioimmunoassay developed by Foster and Dunn (1974) and modified by Redding et al. (1984). Circulating lactic acid concentration was determined using a method modified from Passonneau (1974) by D. Farnsworth for the spectrophotometer at the Oregon Cooperative Fishery Research Unit. Concentrations of plasma glucose were assayed by the *O*-toluidine method outlined by Wedemeyer and Yasutake (1977). Hepatocyte glucose concentrations were determined enzymatically according to the method of Bergmeyer (1983). Liver glycogen content was determined according to Moon et al. (1989).

Statistical Methods

The data were subjected to a one-way analysis of variance. Where *F*-values indicated significance ($p < 0.05$), means were compared using Tukey's test. A computer program (McPherson 1985) was used to analyze binding data by subtracting NSB from total binding to get specific binding and obtain dissociation constants (K_d) and maximum binding sites (B_{max}) based on Scatchard plot analyses (Scatchard 1949).

Results

Continuous swimming of food-deprived coho salmon did not change plasma cortisol or lactate levels relative to those of resting, fed fish. Plasma glucose levels were significantly higher in the exercised group (Table 1). Liver cytosol in resting, fed coho salmon showed saturable GR activity (Fig. 1A), and the Scatchard analysis indicated a high-affinity (K_d = 2.75 nM), low-capacity (B_{max} = 61.1 fmol·mg protein^{-1}) binding site for cortisol (Fig. 1B). There were no significant effects of the com-

TABLE 1. Plasma cortisol, lactate, and glucose concentrations in resting, fed and food-deprived juvenile coho salmon continuously swimming at 1 bl·s^{-1} for 3 wk. Values are shown as mean ± SEM (n); *significantly different from the resting fish ($p < 0.05$; ANOVA).

	Resting, fed	Swimming, unfed
Cortisol (nM)	8.26 ± 2.5 (8)	16.8 ± 3.9 (8)
Lactate (mM)	2.60 ± 0.2 (8)	2.48 ± 0.21 (8)
Glucose (mM)	3.29 ± 0.08 (8)	3.69 ± 0.1 (8)*

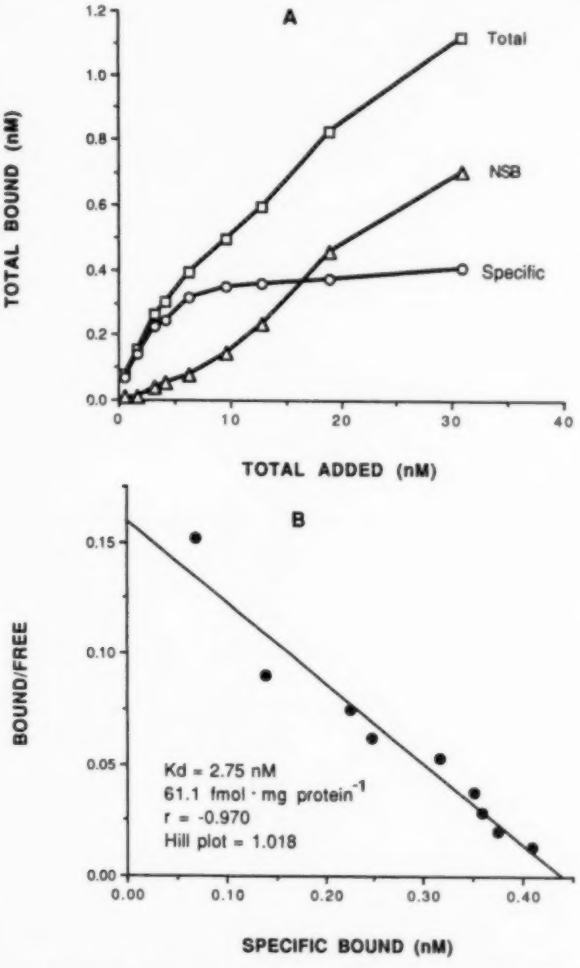


FIG. 1. Binding of [^{3}H] triamcinolone acetonide to liver cytosol from resting, fed coho salmon. (A) Total, specific, and nonspecific (NSB) binding of various concentrations of ligand to 0.20 mL of liver cytosol containing 7.2 mg protein·mL^{-1}. (B) Scatchard plot of data in Fig. 1A with accompanying dissociation constant (K_d), maximum number of receptors, and correlation coefficient (r). Bound/free is the concentration of specifically bound ligand divided by the concentration of free ligand at each concentration of radiolabeled ligand added. The Hill plot value expresses the slope of the line of a hill plot of data in Fig. 1A.

TABLE 2. Changes in hepatosomatic index (HSI), liver glycogen concentration, and hepatic enzyme activities (units (μmol·min^{-1})·g wet mass^{-1}) in resting, fed and food-deprived, continuously swimming coho salmon. Values are shown as mean ± SEM (n); *significantly different from the resting group ($p < 0.05$; ANOVA).

	Resting, fed	Swimming, unfed
HSI	1.26 ± 0.06 (4)	0.94 ± 0.07 (4)*
Glycogen (μmol·g^{-1})	166.1 ± 47.2 (4)	130.7 ± 17.4 (4)
Total GPase	0.50 ± 0.07 (4)	0.49 ± 0.05 (4)
% GPase *a*	28.1 ± 5.4 (4)	24.5 ± 3.1 (4)
Total GSase	1.14 ± 0.15 (4)	1.43 ± 0.26 (4)
% GSase *a*	50.0 ± 10.2 (4)	35.9 ± 9.6 (4)
PK	2.93 ± 0.3 (4)	3.05 ± 0.4 (4)
PK activity ratio	0.03 ± 0.008 (4)	0.02 ± 0.007 (4)
LDH	164.2 ± 11.1 (4)	191.9 ± 23.9 (4)

TABLE 3. Initial hepatocyte glycogen content and percent glycogen breakdown per hour (% HGB · h^{-1}) in resting, fed and food-deprived, continuously swimming coho salmon. Values are shown as mean ± SEM (n);* significantly different from the resting fish ($p < 0.05$; ANOVA).

	Resting, fed	Swimming, unfed
Glycogen (μmol·g^{-1})	75.2 ± 12.8 (4)	31.7 ± 5.4 (4)*
% HGB·h^{-1}	21.0 ± 2.8(4)	7.0 ± 2.3 (4)*

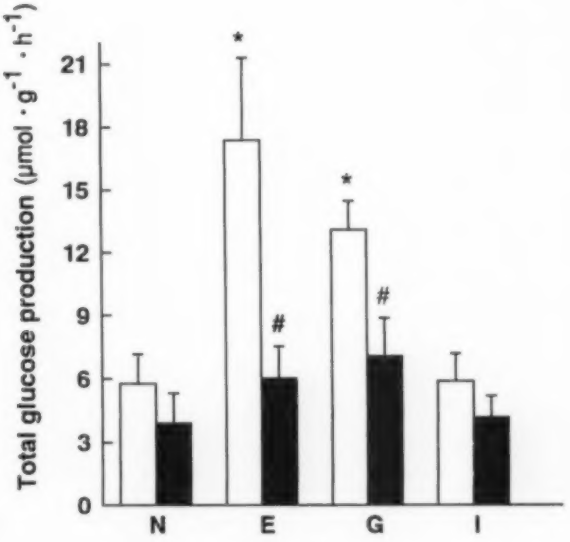


FIG. 2. Total glucose production by hepatocytes isolated from resting, fed (open bar) or food-deprived, continuously swimming (solid bar) coho salmon incubated with no hormone (N), epinephrine (E; 10^{-5} M), glucagon (G; 10^{-8} M), or insulin (I; 10^{-8} M). Values are given as mean ± SEM ($n = 4$); *significantly different from the control (N); #significantly different from the resting, fed fish ($p < 0.05$; ANOVA).

bination of fasting and continuous swimming on either the affinity (2.0 ± 0.2 nM for resting, fed and 1.5 ± 0.1 nM for swimming, unfed) or the capacity of cortisol receptors (56.8 ± 9.8 fmol · mg protein^{-1} for resting, fed and 38.2 ± 7.0 fmol · mg protein^{-1} for swimming, unfed) in the liver cytosol when compared with resting fish.

TABLE 4. Percentage of glucose production that could be explained by glycogen breakdown (GB/GP) in hepatocytes isolated from resting, fed and food-deprived, continuously swimming coho salmon incubated with no hormone (control), epinephrine (10^{-5} M), glucagon (10^{-8} M), or insulin (10^{-8} M). Values shown were obtained by dividing data from Fig. 3 by data from Fig. 2.

	GB/GP	
	Resting	Swimming
Control	100	59
Epinephrine	80	43
Glucagon	78	65
Insulin	100	0

The HSI was significantly lower in the exercised fish than in the resting fish (Table 2). Continuous swimming coupled with food deprivation had no significant effect on liver glycogen content or the activities of enzymes involved in hepatic glycogen metabolism (GPase, GSase, PK, or their phosphorylated states) and LDH activity when compared with the resting, fed fish (Table 2). Even though liver glycogen was unaffected (Table 2), hepatocyte glycogen content and glycogen breakdown per hour (as a percentage of total glycogen in hepatocytes) in the continuously swimming, food-deprived group were significantly lower than in the resting, fed fish (Table 3).

There was no significant difference between the rates of total glucose production (without hormone) by hepatocytes from the two groups (Fig. 2). Incubation of hepatocytes with epinephrine, glucagon, or insulin did not increase total glucose production in the continuously swimming, food-deprived fish. However, in the resting, fed fish, both epinephrine and glucagon increased total glucose production two to three times above the control (no hormone). These values were significantly higher than those for the exercised fish (Fig. 2). Insulin had no effect on total glucose production in the present study (Fig. 2).

Glycogen breakdown in the absence of hormones, however, was significantly different between the two groups (Fig. 3). The higher glycogen breakdown in the resting fish is consistent with a higher glycogen content (Table 3) and could account for all of the glucose produced (Fig. 2). Glycogen breakdown accounts for only 59% of the glucose produced by the hepatocytes from the continuously swimming, food-deprived group (Table 4). Incubation with hormones did not significantly affect glycogen breakdown from the control (no hormone) in the resting, fed fish, and most if not all glucose produced in this group can be accounted for by glycogen breakdown. Epinephrine and glucagon did not alter glycogen breakdown in the exercised, food-deprived fish whereas insulin significantly decreased glycogen breakdown (enhanced deposition).

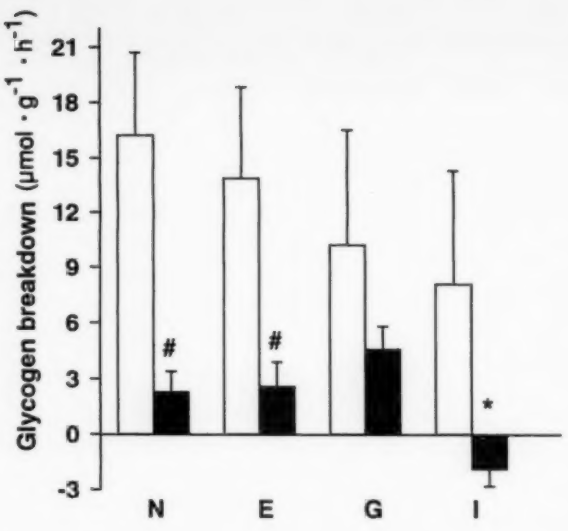


FIG. 3. Total glycogen breakdown by hepatocytes isolated from resting, fed (open bar) or food-deprived, continuously swimming (solid bar) coho salmon incubated with no hormone (N), epinephrine (E; 10^{-5} M), glucagon (G; 10^{-8} M), or insulin (I, 10^{-8} M). Values are given as mean ± SEM ($n = 4$); *significantly different from the control (N); #significantly different from the resting, fed fish ($p < 0.05$; ANOVA).

Discussion

Our data indicate that the potential for glycogen breakdown by hepatocytes is lower in food-deprived, continuously swimming coho salmon. One mechanism operating to conserve glycogen content is a decrease in the sensitivity of hepatocytes to catabolic hormones (e.g., epinephrine and glucagon) and an increase in the sensitivity to anabolic hormones (e.g., insulin) (Fig. 2, 3; Table 4).

Several studies have shown that salmonids protect liver glycogen content during periods of fasting (see Sheridan and Mommsen 1991) and migration (Mommsen et al. 1980; French et al. 1983). This protection is achieved by mobilizing protein and lipid reserves and converting amino acids to glycogen by gluconeogenesis (Cowey et al. 1977; Mommsen et al. 1980; French et al. 1981, 1983; Sheridan and Mommsen 1991). Pancreatic hormones are also known to play an important role in gluconeogenesis (Cowey et al. 1977; Plisetskaya 1990; Mommsen and Plisetskaya 1991), and any subtle alteration in plasma profiles of these hormones may be a factor in energy repartitioning (Moon et al. 1989; Sheridan and Mommsen 1991). The results of the present study clearly indicate that in addition to changes in plasma hormone titre, the target tissue responsiveness to hormones is an important factor operating to protect against excessive liver glycogen mobilization.

Hormonal control of glycogen metabolism is not well understood in fish. Several hormones have been implicated in glycogen metabolism associated with stress and food deprivation in salmonids (Wright et al. 1989; Vijayan et al. 1990; Sheridan and Mommsen 1991; Vijayan and Moon 1992). Also, the prior nutritional state of the animal may influence glycogen metabolism (Vijayan and Moon 1992). In vitro studies with hepatocytes have shown that epinephrine and glucagon increase glucose production by both glycogenolysis and gluconeogenesis (Mommsen et al. 1988; Mommsen and Moon 1990; Brighenti et al. 1991). Our results support the role of these hormones in enhancing total glucose production in resting coho salmon, with glycogenolysis accounting for at least 80% of the glucose produced (Table 4).

In continuously swimming, food-deprived fish, epinephrine and glucagon did not increase glucose production above that of the control (no hormone) (Fig. 2). Thus, it seems that these cells have a lower responsiveness to these hormones in the exercised fish. These changes in responsiveness could be due either to prior exposure to the hormones (desensitization, down regulation) or to cellular changes associated with energy balance and repar-

titioning of energy reserves. A similar decrease in responsiveness to the glycolytic action of glucagon on liver slices has been reported for fasted chinook salmon (Klee et al. 1990). This decrease in responsiveness may be an adaptive strategy to conserve cellular glycogen.

The lower hepatocyte glycogen in the exercised group may also be partly responsible for the lower glycogen breakdown (Table 3), as previous studies have shown a direct correlation of glycogen content with total glycogen breakdown in coho salmon hepatocytes (Mommsen 1986). Thus, it is possible that glycogen content in the hepatocyte in itself might be exerting control on hepatic glycogenolysis (Mommsen 1986). It is not known if this effect is either direct or indirect through alterations in hepatocyte responsiveness. Further work is required before the process can be clearly explained.

Hepatocytes of continuously swimming, food-deprived coho salmon responded differently to insulin and countered the glycogen breakdown seen in the control (no hormone) hepatocytes (Fig. 2, 3; Table 4). This anabolic effect of insulin, as shown by an increased glycogen content in the hepatocytes (Fig. 3), has been reported before in coho salmon (Plisetskaya et al. 1984). However, no such effect of insulin was evident in the resting, fed fish, suggesting that the nutritional state of the animal and energy requirements may also determine hepatic responsiveness to insulin.

A gluconeogenic effect of insulin has been reported previously in fish hepatocytes (see Foster and Moon 1989). Also, several studies suggested an energy repartitioning role for insulin, which is in accordance with the present study (Plisetskaya 1990; Mommsen and Plisetskaya 1991). Cowey et al. (1977) showed that in normally fed fish, insulin reduced gluconeogenesis whereas enhanced gluconeogenesis has been attributed to insulin action in salmon (anorexic) during spawning migration (see Plisetskaya 1990). Thus, it appears that in fish with a negative energy balance the anabolic effect of insulin may be primarily aimed at maintaining liver glycogen content. This is probably achieved by enhanced gluconeogenesis, where C3 precursors are directed towards glycogen synthesis (Foster and Moon 1987, 1989). In fed fish with a positive energy balance, the anabolic effect of insulin directs amino acids into skeletal muscle and liver protein (Mommsen and Plisetskaya 1991). Insulin may be acting synergistically with other glucoregulatory hormones in this energy partitioning process (Foster and Moon 1987). In the present study, the changes in responsiveness to insulin seen in the exercised fish occur at the cellular level possibly through alterations either in the receptors or in the signal transduction pathway (Foster and Moon 1990).

There was no change in plasma lactate levels in the swimming fish, indicating that the metabolism was maintained aerobically during swimming (Table 1). The higher plasma glucose levels in the swimming fish indicate an increased metabolic rate that is probably met by enhanced gluconeogenesis. Gluconeogenic activity was found to increase in the liver of trained fish (Mosse 1980), as were blood glucose levels and phosphoenolpyruvate carboxykinase activities (Johnston and Moon 1980). Pancreatic hormones have been implicated in the mobilization of lipids and proteins and the provision of glucose during long-term fasting of coho salmon (Sheridan and Mommsen 1991). It is likely that these hormones, either alone or in concert with other glucoregulatory hormones, maintained plasma glucose levels in our fish. The lack of changes in hepatic enzyme activities (Table 2) in the present study does not support enhanced hepatic glycogen mobilization for the maintenance of plasma glucose. Thus, gluconeogenesis may be a contributing factor to the plasma glucose levels in the food-deprived, continuously swimming coho salmon.

Cortisol may also enhance gluconeogenesis by the breakdown of proteins and lipids in teleosts (Sheridan 1986; Vijayan et al. 1991). Elevated cortisol levels during stress help the fish to adapt metabolically by mobilizing energy reserves and increasing plasma glucose levels (Vijayan and Moon 1992). Cortisol has also been shown to mobilize liver glycogen in teleosts (Vijayan et al. 1991; Vijayan and Leatherland 1992). There was no change in plasma cortisol levels in the present study (Table 1), suggesting that our fish were unstressed. However, the clearance of cortisol may have been increased, thereby maintaining low plasma cortisol levels in the exercised fish (Vijayan and Leatherland 1990). However, that seems unlikely, as there was no change in the target tissue pool of cytosolic cortisol binding sites in coho salmon. Cortisol binding to liver (Pottinger 1990) and gill (Maule and Schreck 1991) cytosol appears to decrease with chronic exposure to stress or cortisol treatment. Although there was a trend toward a lower number of GR in fasted, exercised fish, the absence of any significant changes in the affinity or capacity of GR in liver cytosol suggests that those fish were not exposed to elevated cortisol concentrations. This might be an adaptive strategy whereby excessive mobilization of glycogen is avoided (Vijayan and Moon 1992). However, when exposed to stressful situations, increases in cortisol might result in immediate mobilization of energy resources to cope with the increased energy demand associated with stress (Vijayan and Moon 1992).

In conclusion, the results of this study indicate that continuously swimming fish deprived of food (as in migration) have developed strategies to conserve hepatic glycogen or prevent excessive mobilization. This may be brought about by altering the responsiveness of hepatocytes to hormonal stimulation. Specifically, in swimming fish the responsiveness to glucagon and epinephrine (catabolic hormones) appears to be reduced whereas the sensitivity to insulin (anabolic hormone) is elevated to replenish hepatic glycogen stores compared with resting, fed fish. It is likely that alterations in the profiles of glucoregulatory hormones and in target tissue responsiveness play an important role in conserving glycogen during long periods of migration. The maintenance of hepatic glycogen stores may be important to cope metabolically with acute or chronic stressors. Cortisol may not be playing a significant role in the glycogen mobilization process in the food-deprived, continuous swimming group, but may be important for immediate glycogen mobilization during stress (such as facing obstacles during migration).

Acknowledgements

Thanks are extended to Prof. Steve Perry for reviewing the manuscript. The authors thank Dan Farnsworth for his excellent technical assistance, Cameron Sharpe, Logi Johansson, Choo-Guan Yeoh, Iris Knoble, and all the other people in C.B.S.'s laboratory for their help, and Rob Chitwood for maintaining the experimental fish. This study was supported by Natural Sciences and Engineering Research Council (NSERC) grant No. A6944 to T.W.M. and an NSERC postdoctoral fellowship to M.M.V.

References

BEAMISH, F.W.H. 1964. Influence of starvation on standard and routine oxygen consumption. Trans. Am. Fish. Soc. 93: 103–107.

BERGMEYER, H.U. [ED.] 1983. Methods of enzymatic analysis. Vol. VI. 3rd ed. Verlag Chemie, Weinheim. p. 163–172 and 582–588.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye binding. Anal. Biochem. 72: 248–252.

BRIGHENTI, L., A.C. PUVIANI, M.E. GAVIOLI, E. FABBRI, AND C. OTTOLENGHI. 1991. Interaction of salmon glucagon, glucagon-like peptide, and epinephrine in the stimulation of phosphorylase a activity in fish isolated hepatocytes. Gen. Comp. Endocrinol. 82: 131–139.

COWEY, C.B., D. KNOX, M.J. WALTON, AND J.W. ADRON. 1977. The regulation of gluconeogenesis by diet and insulin in rainbow trout (*Salmo gairdneri*). Br. J. Nutr. 38: 463–470.

FOSTER, G.D., AND T.W. MOON. 1986. Cortisol and liver metabolism of immature American eels, *Anguilla rostrata* (LeSueur). Fish Physiol. Biochem. 1: 113–124.

FOSTER, G.D., AND T.W. MOON. 1987. Metabolism in sea raven (*Hemitripterus americanus*) hepatocytes: the effects of insulin and glucagon. Gen. Comp. Endocrinol. 66: 102–115.

FOSTER, G.D., AND T.W. MOON. 1989. Insulin and the regulation of glycogen metabolism and gluconeogenesis in American eel hepatocytes. Gen. Comp. Endocrinol. 73: 374–381.

FORSTER, G.D., AND T.W. MOON. 1990. The role of glycogen phosphorylase in the regulation of glycogenolysis by insulin and glucagon in isolated eel (*Anguilla rostrata*) hepatocytes. Fish Physiol. Biochem. 8: 299–309.

FOSTER, L.B., AND R.T. DUNN. 1974. Single-antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. Clin. Chem. 20: 365–368.

FRENCH, C.J., P.W. HOCHACHKA, AND T.P. MOMMSEN. 1983. Metabolic organization of liver during spawning migration of sockeye salmon. Am. J. Physiol. 245: R827–R830.

FRENCH, C.J., T.P. MOMMSEN, AND P.W. HOCHACHKA. 1981. Amino acid utilization in isolated hepatocytes from rainbow trout. Eur. J. Biochem. 113: 311–317.

JOHNSTON, I.A., AND T.W. MOON . 1980. Exercise training in skeletal muscle of brook trout (*Salvelinus fontinalis*). J. Exp. Biol. 87: 177–194.

KLEE, M., C. EILERTSON, AND M.A. SHERIDAN. 1990. Nutritional state modulates hormone-mediated hepatic glycogenolysis in chinook salmon (*Oncorhynchus tshawytscha*). J. Exp. Zool. 254: 202–206.

MAULE, A.G., AND C.B. SCHRECK. 1990. Glucocorticoid receptors in leukocytes and gill of juvenile coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 77: 448–455.

MAULE, A.G., AND C.B. SCHRECK. 1991. Stress and cortisol treatment changed affinity and number of glucocorticoid receptors in leukocytes and gill of coho salmon. Gen. Comp Endocrinol. 84: 83–93.

MAULE, A.G., R.A. TRIPP, S.L. KAATTARI, AND C.B. SCHRECK. 1989. Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). J. Endocrinol. 120: 135–142.

MCPHERSON, G.A. 1985. Kinetics, EBDA, ligand, lowry. A collection of radioligand binding analysis programs. Elsevier Publishers, Amsterdam, The Netherlands.

MOMMSEN, T.P. 1986. Comparative gluconeogenesis in hepatocytes from salmonid fishes. Can. J. Zool. 64: 1110–1115.

MOMMSEN, T.P., C.J. FRENCH, AND P.W. HOCHACHKA. 1980. Sites and patterns of protein and amino acid utilization during the spawning migration of salmon. Can. J. Zool. 58: 1785–1799.

MOMMSEN, T.P., AND T.W. MOON. 1990. Metabolic actions of glucagon-family hormones in teleost hepatocytes. J. Endocrinol. 126: 109–118.

MOMMSEN, T.P., AND E.M. PLISETSKAYA. 1991. Insulin in fishes and agnathans: history, structure, and metabolic regulation. Rev. Aquat. Sci. 4: 225–259.

MOMMSEN, T.P., AND R. SUAREZ . 1984. Control of gluconeogenesis in rainbow trout hepatocytes: role of pyruvate branchpoint and phosphoenolpyruvate–pyruvate cycle. Mol. Physiol. 6: 9–18.

MOMMSEN, T.P., P.J. WALSH, S.F. PERRY, AND T.W. MOON. 1988. Interactive effects of catecholamines and hypercapnia on glucose production in isolated trout hepatocytes. Gen. Comp. Endocrinol. 70: 63–73.

MOON, T.W., G.D. FOSTER, AND E.M. PLISETSKAYA. 1989. Changes in peptide hormones and liver enzymes in the rainbow trout deprived of food for 6 weeks. Can. J. Zool. 67: 2189–2193.

MOSSE, P.R.L. 1980. An investigation of gluconeogenesis in marine teleosts and the effects of long term exercise on hepatic gluconeogenesis. Comp. Biochem. Physiol. 67B: 583–592.

PASSONNEAU, J.F. 1974. Fluorimetric method, p. 1468–1477. *In* H.U. Bergmeyer. [ed.] Methods of enzymatic analysis. 2nd Engl. ed. Academic Press, New York, N.Y.

PASSONNEAU, J.V., AND D.A. ROTTENBERG. 1973. An assessment of methods for measurement of glycogen synthetase activity including a new direct one-step assay. Anal. Biochem. 51: 528–541.

PERRY, S.F., P.J. WALSH, T.P. MOMMSEN, AND T.W. MOON. 1988. Metabolic consequences of hypercapnia in the rainbow trout, *Salmo gairdneri*: β-adrenergic effects. Gen Comp. Endocrinol. 69: 439–447.

PLISETSKAYA, E.M. 1990. Recent studies of fish pancreatic hormones: selected topics. Zool. Sci. 7: 335–353.

PLISETSKAYA, E.M., S. BHATTACHARYA, W.W. DICKHOFF, AND A. GORBMAN. 1984. The effect of insulin on amino acid metabolism and glycogen content in isolated liver cells of juvenile coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol. 78A: 773–778.

POTTINGER, T.G. 1990. The effect of stress and exogenous cortisol on receptor-like binding of cortisol in the liver of rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 78: 194–203.

REDDING, J.M., C.B. SCHRECK, E.K. BIRKS, AND R.D. EWING. 1984. Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in freshwater and during seawater acclimation in yearling coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 56: 146–155.

SCATCHARD, G. 1949. The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51: 660–672.

SHERIDAN, M.A. 1986. Effects of thyroxin, cortisol. growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. Gen. Comp. Endocrinol. 64: 220–238.

SHERIDAN, M.A., AND T.P. MOMMSEN. 1991. Effects of nutritional state on in vivo lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus* kisutch. Gen. Comp. Endocrinol. 81: 473–483.

VIJAYAN, M.M., J.S. BALLANTYNE, AND J.F. LEATHERLAND. 1990. High stocking density alters the energy metabolism of brook charr, *Salvelinus fontinalis*. Aquaculture 88: 371–381.

VIJAYAN, M.M., J.S. BALLANTYNE, AND J.F. LEATHERLAND. 1991. Cortisol-induced changes in some aspects of the intermediary metabolism of *Salvelinus fontinalis*. Gen. Comp. Endocrinol. 82: 476–486.

VIJAYAN, M.M., G.D. FOSTER, AND T.W. MOON. 1993. Effects of cortisol on hepatic carbohydrate metabolism and responsiveness to hormones in the sea raven, *Hemitripterus americanus*. Fish Physiol. Biochem. (In press)

VIJAYAN, M.M., AND J.F. LEATHERLAND. 1990. High stocking density affects cortisol secretion and tissue distribution in brook charr, *Salvelinus fontinalis*. J. Endocrinol. 124: 311–318.

VIJAYAN, M.M., AND J.F. LEATHERLAND. 1992. In vivo effects of the steroid analogue RU486 on some aspects of intermediary and thyroid metabolism of brook charr, *Salvelinus fontinalis*. J. Exp. Zool. 263: 265–271.

VIJAYAN, M.M., AND T.W. MOON. 1992. Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 49: 2260–2266.

WEDEMEYER, G.A., AND W.T. YASUTAKE. 1977. Clinical methods for the assessment of the effects of environmental stress in fish health. U.S. Fish Wild. Serv. Tech. Pap. 89.

WRIGHT, P.A., S.F. PERRY, AND T.W. MOON. 1989. Regulation of hepatic gluconeogenesis and glycogenolysis by catecholamines in rainbow trout during environmental hypoxia. J. Exp. Biol. 147: 169–188.

Relative Contributions of Dietary and Waterborne Copper to Tissue Copper Burdens and Waterborne-Copper Tolerance in Rainbow Trout (*Oncorhynchus mykiss*)

P.A. Miller,[1] R.P. Lanno, M.E. McMaster,[2] and D.G. Dixon[3]

Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1, Canada

Miller, P.A., R.P. Lanno, M.E. McMaster, and D.G. Dixon. 1993. Relative contributions of dietary and waterborne copper to tissue copper burdens and waterborne-copper tolerance in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 50: 1683–1689.

In a 42-d study, rainbow trout (*Oncorhynchus mykiss*) were fed a diet containing either 13 or 684 mg Cu·kg^{-1} and simultaneously exposed to waterborne-Cu concentrations of 5, 32, 55, or 106 µg·L^{-1} (low-Cu diet) or 13, 38, 62, or 127 µg·L^{-1} (high-Cu diet). There were no significant effects on mortality, growth, condition factor, or food conversion efficiency. Elevated dietary Cu increased Cu concentrations in liver ($p < 0.001$), kidney ($p < 0.001$), gill ($p = 0.005$), and digesta ($p < 0.001$). Increasing waterborne-Cu concentrations elevated Cu concentrations in liver ($p = 0.018$) and kidney ($p = 0.002$) but not in gill ($p = 0.930$) or digesta ($p = 0.519$). Waterborne-Cu exposure increased Zn concentrations in liver ($p = 0.025$) but decreased those in kidney ($p = 0.045$). For fish on the high-Cu diet, diet provided 99, 85, and 63% of the Cu in the liver for the 38, 62, and 127 µg·L^{-1} waterborne-Cu treatments, respectively. Based on Cu tolerance (incipient lethal level for Cu), dietary and waterborne Cu partitioned into functionally different compartments. Although both waterborne-Cu ($p < 0.00001$) and dietary-Cu ($p = 0.019$) preexposure increased Cu tolerance, waterborne Cu had a much greater impact.

Au cours d'une étude d'une durée de 42 jours, nous avons soumis des truites arc-en-ciel (*Oncorhynchus mykiss*) à un régime alimentaire renfermant soit 13, soit 684 mg Cu·kg^{-1} et, en même temps, nous les avons exposées à des concentrations aqueuses de Cu de 5, de 32, de 55 ou de 106 µg·L^{-1} (régime à faible teneur en Cu) ou de 13, de 38, de 62 ou de 127 µg·L^{-1} (régime riche en Cu). On n'a observé aucun effet significatif sur la mortalité, la croissance, le coefficient de condition ou l'efficience alimentaire. Le régime riche en Cu s'est traduit par des concentrations accrues de cet élément dans le foie ($p < 0{,}001$), dans les reins ($p < 0{,}001$), dans les ouïes ($p = 0{,}005$) et dans les digestas ($p < 0{,}001$). Un accroissement des concentrations aqueuses de Cu a provoqué une augmentation des concentrations de Cu dans le foie ($p = 0{,}018$) et les reins ($p = 0{,}002$) mais non dans les ouïes ($p = 0{,}930$) ou le digesta ($p = 0{,}519$). L'exposition au Cu en solution aqueuse a augmenté les concentrations de Zn dans le foie ($p = 0{,}025$), mais elle a diminué les concentrations rénales ($p = 0{,}045$). Chez le poisson soumis au régime riche en Cu, ce régime est à l'origine de respectivement 99, 85 et 63 % du Cu retrouvé dans le foie chez les poissons exposés aux concentrations aqueuses de Cu de 38, de 62 et de 127 µg·L^{-1}. Si on considère la tolérance au Cu (concentration où ce dernier commence à être mortel), le Cu d'origine alimentaire et le Cu aqueux se sont séparés dans des compartiments fonctionnels différents. Si l'exposition préalable au Cu en solution aqueuse ($p < 0{,}00001$) et au Cu alimentaire ($p = 0{,}019$) a accru la tolérance à cet élément, l'effet de loin le plus grand provient du Cu en solution aqueuse.

Received September 14, 1992
Accepted March 5, 1993
(JB631)

Reçu le 14 septembre 1992
Accepté le 5 mars 1993

Although water quality objectives use aqueous metal levels to protect fish populations from impact (CCREM 1987), there is increasing concern over the potential availability, largely through the diet, of metals contained in contaminated sediments. At the present time, the relative importance of the two routes remains unclear. Although it has been demonstrated that waterborne exposure plays a dominant role in the availability of a number of metals including Cd (Part and Svanberg 1981) and Pb (Hodson et al. 1978), it has been suggested that diet is a much more significant contributor for nutritionally essential metals such as Cu and Zn (Spry et al. 1988).

Although it is apparent that Cu and Zn can be assimilated from either diet or water (Benoit 1975; Holcombe et al. 1979; Lanno et al. 1985), the interaction between the two routes has not been widely studied. We previously reported (Miller et al. 1992) on the relationship of Cu and Zn concentrations in white sucker (*Catostomus commersoni*) tissues to concentrations of those metals in water, sediment, and benthic invertebrates (diet) for six Northern Ontario lakes contaminated with mixed-metal mine waste. For both metals, the concentrations in water were consistently a far better predictor of fish-tissue contamination than the concentrations in either sediment or invertebrates. Unfortunately, design constraints imposed by the field study made it impossible to determine the relative contributions of the two exposure routes to the tissue levels in the fish. The present

[1]Current address: CanTox Inc., Suite 308, 2233 Argentia Road, Mississauga, ON L5N 2X7, Canada.

[2]Current address: Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada.

[3]Author to whom correspondence should be addressed.

laboratory study was therefore initiated to investigate the relative contributions of dietary and waterborne Cu to tissue Cu levels in rainbow trout (*Oncorhynchus mykiss*) under more controlled conditions.

In addition to potential differences in availability, we also wished to determine whether there was any indication of differences in the impacts of the Cu assimilated from the two exposure routes. Studies with both Cu (Dixon and Sprague 1981) and Zn (Sinley et al. 1974) have demonstrated elevated tolerance, as indicated by an increased incipient lethal level, following chronic waterborne preexposure to these metals. Similar studies with dietary-metal preexposure have not been documented. Luoma (1977) suggested that if one population of a species is more tolerant of a toxicant than others, this constitutes strong evidence that the concentration of the toxicant at the site of the tolerant population was sufficient to elicit biological effects. To this end, we assessed the Cu tolerance of the fish from each of the treatments at the end of the uptake study.

Materials and Methods

We used a factorial design to determine the effects of dietary and waterborne Cu on tissue metal levels and Cu tolerance in rainbow trout. Fish were reared for 42 d on one of two diets that varied in Cu concentration (nominally 0 or 665 mg $Cu \cdot kg^{-1}$). They were simultaneously exposed to one of four levels of waterborne Cu (nominally 0, 30, 60, or 120 $\mu g \cdot L^{-1}$). Since we were concerned that a strong and overt Cu toxicosis could interfere with Cu metabolism and kinetics in the fish, the levels of Cu in both the water and the diet were chosen to fall within the range causing sub- to low-chronic toxicity (Dixon and Sprague 1981; Lanno et al. 1985). The experiment was carried out in triplicate for a total of 24 groups (two dietary-Cu levels × four waterborne-Cu levels × three replicates). Treatment effects on fish were assessed in terms of total length, wet weight, condition factor, and food conversion efficiency. Cu concentrations in liver, kidney, gill, vertebral bone, and digesta (contents only of the digestive tract) were also determined. These tissues were chosen as reliable indicators of metal contamination, based on our previous field study (Miller et al. 1992). Since Cu and Zn are known to be subject to cometabolism (Knox et al. 1984; Cousins 1985), Zn levels in liver and kidney tissue were also measured. At the end of the uptake study, fish from each treatment were subjected to acute-lethality toxicity tests to determine their tolerance to waterborne Cu.

The practical trout diets used during the study (Table 1) were prepared at the Department of Nutritional Sciences, University of Guelph, Guelph, Ont. Copper sulphate ($CuSO_4 \cdot 5H_2O$), oven-dried to a constant weight and powdered by mortar and pestle, was added to one half of the formulation during preparation to achieve the concentration required for the high-Cu diet. The diets were steam-pelleted (California Pellet Mill, San Francisco, Calif.), air-dried at room temperature, crumbled, sieved to remove fines, and stored in polyethylene bags at −20°C pending use. The mean (SE, *n*) assayed Cu concentrations in the low- and high-Cu diets were 13 (0.3, 3) and 684 (15.6, 3) mg $Cu \cdot kg^{-1}$, respectively. The low-Cu diet was not Cu deficient (Lanno et al. 1985).

Juvenile rainbow trout, with an initial mean weight of approximately 8 g, were obtained from Spring Valley Trout Farm, New Dundee, Ont. They were held for 2 wk at 11°C in an 800-L aerated fibreglass tank that received a continuous flow

TABLE 1. Formulation of the practical experimental diet used during the 42-d study on the availability of Cu to rainbow trout.

Ingredient	Percent by weight
Fish meal (capelin, 60% crude protein)	38
Soybean meal (49% crude protein)	25
Wheat middlings (18% crude protein)	22
Fish oil	12
Mineral premix[a]	1
Vitamin premix[b]	2

[a]Providing (per kilogram of diet): NaCl (0.015% I), 5000 mg; $FeSO_4$, 300 mg; $MnSO_4 \cdot H_2O$, 126 mg; $CoCl_2 \cdot 6H_2O$, 20 mg; KI, 6 mg; $ZnSO_4 \cdot H_2O$, 15 mg; wheat middlings (filler), 4533 mg.

[b]Providing (per kilogram of diet): vitamin (VIT) A (retinyl palmitate), 4000 IU; VIT D_3 (cholecalciferol), 1000 IU; VIT E (*d*,1-α-tocopherol acetate), 300 mg; VIT K (sodium menadion), 27 mg; thiamin NO_3, 55 mg; riboflavin, 60 mg; *d*-Ca-pantothenate, 165 mg; biotin, 0.5 mg; folate, 10 mg; VIT C (ascorbic acid), 400 mg; choline chloride, 2000 mg; VIT B_{12} (0.5% cobalamin), 30 mg; niacin, 250 mg; pyridoxine HCl, 30 mg; DL-methionine, 3000 mg; butylated hydroxytoluene, 250 mg; wheat middlings (filler), 8423 mg.

(2 $L \cdot min^{-1}$) of groundwater. During this time, the fish were fed to satiation twice daily with a nutritionally complete commercial diet (Juvenile Trout Grower, 52% protein, formulation No. 810111, Martin's Feed Mills, Elmira, Ont.). During acclimatization, and the subsequent uptake and tolerance exposures, the photoperiod was maintained at 16 h light : 8 h dark with 0.5 h of artificial dusk and dawn included in the light portion of the cycle.

After acclimatization the fish were distributed to 24 rectangular self-cleaning polyethylene tanks (70 fish per tank). Each 64-L tank was aerated and received a continuous flow (600 $mL \cdot min^{-1}$) of water. After an additional week, during which the fish were fed the commercial diet, the uptake study was initiated by switching the fish to the experimental diet and starting the waterborne-Cu exposure. Mariotte bottles (Leduc 1966) dispensed Cu stock solutions (prepared from C.P. grade $CuSO_4 \cdot 5H_2O$) into the dilution water streams of the 18 tanks used for the waterborne-Cu treatments to achieve the required design concentrations. Water samples for the determination of Cu concentration were taken from each of the 24 tanks daily up to day 21 and every second day thereafter. The samples were acidified to 1% with analytical grade HNO_3 (15.9 M) and stored in linear polyethylene bottles pending analysis.

The fish were fed to satiation three times daily during the study. Feed consumption was monitored visually to confirm that all feed presented to the fish was consumed. The daily food consumption (weight) was recorded on a per tank basis. Although the tanks were self-cleaning, they were siphoned of feces after each feeding. The total wet weight of the fish in each tank was determined biweekly. The fish were counted into a polyethylene colander held in water. The colander was then weighed with and without fish to give (by difference) the total fish biomass. Food conversion efficiencies over the last 2 wk of the experiment were calculated (on a per tank basis) as (tank biomass$\cdot n^{-1}$)/(weight of ration consumed$\cdot n^{-1}$) where n was the number of fish in the tank.

During the uptake study, and the subsequent tolerance tests, water temperature and pH were determined daily. Total hardness (EDTA titration; APHA 1980), total dissolved oxygen (Winkler titration; APHA 1980), and alkalinity (HCl titration; APHA 1980) were determined periodically from randomly selected

TABLE 2. Mean (SE) wet weight, total length, condition factor (k), and food conversion efficiency (FCE) shown by rainbow trout at the end of a 42-d exposure to dietary and/or waterborne Cu. The mean (SE) assayed dietary-Cu and waterborne-Cu concentrations during the study are also given. Except where indicated, all means have a sample size of 12. There were no significant ($p > 0.05$) treatment effects for any of the biological factors. Within columns for dietary and waterborne Cu concentration, values followed by the same letter are not significantly different ($p > 0.05$).

		Biological factors			
Assayed Cu concentration in diet ($\mu g \cdot g^{-1}$)[a]	Assayed Cu concentration in water ($\mu g \cdot L^{-1}$)[b]	Wet weight (g)	Total length (mm)	k[c]	FCE[d]
13 (0.3) a	5 (1) a	20.2 (1.5)	123 (3)	1.07 (0.02)	1.5 (0.9)
	32 (1) b	23.1 (2.0)	127 (3)	1.08 (0.01)	1.6 (0.7)
	55 (2) c	27.7 (1.6)	137 (2)	1.05 (0.02)	0.8 (0.3)
	106 (4) d	25.4 (2.4)	131 (3)	1.09 (0.02)	0.7 (0.2)
684 (16) b	13 (1) e	23.7 (1.5)	130 (3)	1.08 (0.02)	0.7 (0.2)
	38 (2) f	25.4 (1.6)	132 (2)	1.07 (0.01)	0.8 (0.1)
	62 (2) g	24.1 (2.0)	130 (4)	1.10 (0.03)	1.8 (0.9)
	127 (5) h	20.2 (1.4)	123 (3)	1.08 (0.02)	1.1 (0.4)

[a]Sample size = 3.
[b]Sample size = 71–77.
[c]$k = 100(\text{weight} \cdot \text{length}^{-3})$ with the weight and length of the fish in grams and centimetres, respectively.
[d]FCE was calculated on a per tank basis over the last 2 wk of the study as (tank biomass$\cdot n^{-1}$)/(weight of ration consumed$\cdot n^{-1}$) where n was the number of fish in the tank. Sample size = 3.

tanks. The mean (SE, n) water conditions were as follows: temperature, 14.0 (0.6, 42)°C; total hardness, 380 (4, 11) $mg \cdot L^{-1}$ as $CaCO_3$; alkalinity, 280 (3, 11) $mg \cdot L^{-1}$ as $CaCO_3$; pH, 7.6 (0.2, 42); dissolved oxygen, consistently >90% saturation. A detailed characterization of the groundwater supplying our laboratory is given in Kevan and Dixon (1991).

At the end of the 42-d exposure, four fish from each replicate (12 per treatment) were killed by an overdose of tricaine methanesulphonate (MS 222, Syndel Laboratories, Vancouver, B.C.). Total length and wet weight were recorded for each fish. Condition factor (k) was calculated as $100(\text{weight} \cdot \text{length}^{-3})$ with the weight and length of the fish in grams and centimetres, respectively. An additional six fish per treatment (two per replicate) were killed as above, and samples of liver, kidney, gill, and vertebral bone, as well as the entire digesta, were excised, placed in polyethylene vials, and stored at −20°C pending metals analysis.

The Cu tolerance of the fish in each treatment was assessed with acute-lethality toxicity tests that exposed 10 fish per concentration to nominal Cu levels of 0, 270, 410, 610, 940, and 1450 $\mu g \cdot L^{-1}$. Due to the limited number of fish, the toxicity tests were carried out in duplicate (as opposed to triplicate) for a total of 16 tests (two diets × four waterborne-Cu concentrations × two replicates). The tests were completed using continuous-flow exposure tanks and holding conditions identical to those of the feeding trial. Due to the limited availability of tanks, and in order to complete all of the tests simultaneously, fish from different preexposure treatments (identified by distinctive fin clips) were mixed together during exposure. Grand mean (SE, n) assayed Cu concentrations for the five exposure levels, based on daily sampling of each tank, were 220 (47, 40), 322 (47, 39), 510 (90, 40), 792 (85, 31), and 1131 (113, 20) $\mu g \ Cu \cdot L^{-1}$, representing 78–84% of nominal. The fish were not fed during the toxicity tests, which continued for 144 h.

Metal levels in water, tissue, and diet samples were determined by flame-volatilization atomic absorption spectrophotometry after appropriate preparation. Water samples were analyzed by direct aspiration. Tissue and diet samples were oven-dried for 60 h at 60°C, weighed, and transferred to 125-mL Erlenmeyer flasks. Mixed acid (1:8 (v/v) 9 M H_2SO_4 – 15.9 M HNO_3) was added to the flasks at a rate of 5 mL per 0.3–0.4 g of sample. The samples were refluxed using small glass funnels at 200–220°C for approximately 1 h with the addition of 5 mL of hydrogen peroxide (30%) and then, prior to analysis, diluted to either 25 or 50 mL with distilled water. All analyses were completed with a Perkin-Elmer 4000 atomic absorption spectrophotometer equipped with an impact bead to increase the sensitivity and lower the detection limit (detection limit: Cu, 1 $\mu g \cdot L^{-1}$, Zn, 1 $\mu g \cdot L^{-1}$). Standard curves of Cu and Zn concentration were constructed using commercially available certified stock solutions. Analytical blanks appropriate to the design were completed in conjunction with the samples. The method for determining the levels of Cu and Zn in tissues was verified with National Bureau of Standards bovine liver (lot 1577a). Our analytical method yielded mean (SE, n) values of 150.7 (5.0, 4) $\mu g \ Cu \cdot g^{-1}$ and 117.0 (2.0, 4) $\mu g \ Zn \cdot g^{-1}$, representing 95.4 and 95.1% recovery, respectively.

Treatment and replicate effects on Cu and Zn concentration in tissues as well as on Cu tolerance were assessed by factorial analysis of variance (ANOVA) followed by Tukey's comparison of means. ANOVA was also used to test for potential impacts of dietary-Cu concentration on waterborne-Cu concentration. All data sets were tested for normality and homogeneity of variance. The data for Cu and Zn concentration in liver and kidney tissue, and Cu concentration in gill tissue, were log-transformed to obtain homogeneity of variance prior to ANOVA. There were no replicate effects in any case ($p > 0.25$ and usually >0.50). Statistical analyses were conducted using personal computer based software (SYSTAT 5.0; Systat Inc., Evanston, Ill.). LC50s were calculated by trimmed Spearman–Karber analysis (Hamilton et al. 1977) using mortality and mean assayed Cu concentration. No correction for control mortality was required. Since all of the

TABLE 3. Geometric mean (SE) tissue concentrations ($n = 6$) of Cu and Zn in rainbow trout at the end of a 42-d exposure to dietary and/or waterborne Cu. Within columns, values followed by the same letter are not significantly different ($p > 0.05$). There were no significant dietary by waterborne treatment-interaction effects.

Dietary Cu concentration ($\mu g \cdot L^{-1}$)[a]	Waterborne Cu concentration ($\mu g \cdot L^{-1}$)[a]	Tissue Cu concentration ($\mu g \cdot g^{-1}$)				Tissue Zn concentration ($\mu g \cdot g^{-1}$)	
		Liver	Kidney	Gill	Digesta	Liver	Kidney
13	5	147 (17) a	11 (4) ab	7 (1) a	8 (1) a	60 (4) a	91 (3) a
	32	161 (11) a	7 (3) a	7 (1) a	9 (1) a	79 (2) ab	94 (6) a
	55	215 (17) ab	18 (2) b	8 (1) a	8 (1) a	77 (3) b	102 (7) a
	106	310 (31) b	17 (2) b	9 (1) a	12 (2) a	80 (5) b	79 (8) a
684	13	466 (46) c	29 (6) cd	24 (9) a	346 (44) b	62 (7) a	88 (7) a
	38	379 (82) c	18 (4) c	15 (4) a	222 (45) b	72 (4) ab	104 (8) a
	62	579 (76) cd	47 (10) d	12 (3) a	229 (50) b	84 (8) b	97 (8) a
	127	732 (42) d	62 (23) d	16 (7) a	263 (58) b	78 (3) b	89 (11) a

[a]See Table 2 for SEs and sample sizes.

acute toxicity tests achieved thresholds of mortality, the LC50s constitute incipient lethal levels.

The relative contributions of dietary versus waterborne Cu to liver-Cu levels were calculated using the method of Spry et al. (1988) with modifications. At control waterborne-Cu and high dietary-Cu concentrations, uptake from the water was assumed to be negligible, and the entire liver-Cu burden above control–control values (neither dietary-Cu nor waterborne-Cu supplementation) was assumed to be of dietary origin. For each water level above control, the difference between the initial and final liver burden minus the same difference at control Cu concentrations for that dietary level was considered to be the absolute amount contributed by the water. This contribution was expressed as a percentage of the total liver burden.

Results

Factorial ANOVA demonstrated that there was a significant ($p < 0.005$) interaction between dietary-Cu and waterborne-Cu concentration (Table 2); waterborne-Cu concentrations in the treatments receiving the high-Cu diet were significantly higher than those receiving the low-Cu diet, presumably because some of the $CuSO_4$ in the diet was leaching into the water. Although this interaction was highly significant statistically, subsequent ANOVAs of dietary-Cu and waterborne-Cu effects on weight, length, condition factor, food conversion efficiency, and tissue-metal levels (see below) showed no interactions between treatments. Had the impact of diet on waterborne-Cu concentration been highly relevant biologically, the effect would have shown as significant interactions in these subsequent ANOVAs.

There were no substantial signs of overt Cu toxicity during the 42-d study. No mortality occurred and there were no significant dietary-Cu or waterborne-Cu effects on wet weight ($p = 0.91$ and 0.33, respectively), length ($p = 0.90$ and 0.17), condition factor ($p = 0.95$ and 0.78), or food conversion efficiency ($p = 0.86$ and 0.89) (Table 2).

Dietary Cu significantly increased Cu concentrations in the liver ($p < 0.001$), kidney ($p < 0.001$), gill ($p = 0.005$), and digesta ($p < 0.001$) (Table 3). Waterborne Cu also increased Cu concentrations in the liver ($p = 0.018$). Tukey's analysis revealed that the highest water concentration had a significant impact on liver-Cu concentration relative to the lower waterborne concentrations. There were also elevated Cu levels

TABLE 4. 144-h LC50s for rainbow trout preexposed to dietary and/or waterborne Cu for 42 d. For the column of mean (SE) treatment LC50s, values followed by the same letter are not significantly different ($p > 0.05$).

Dietary Cu concentration ($\mu g \cdot g^{-1}$)[a]	Waterborne Cu concentration ($\mu g \cdot L^{-1}$)[a]	144-h Cu LC50 ($\mu g \cdot L^{-1}$)		
		Replicate 1	Replicate 2	Mean (SE)
13	5	270	270	270 (—) a
	32	270	270	270 (—) a
	55	270	327	299 (29) a
	106	523	566	545 (11) b
684	13	333	320	322 (7) a
	38	319	348	334 (15) a
	62	270	270	270 (—) a
	127	569	590	580 (11) b

[a]See Table 2 for SEs and sample sizes.

($p = 0.002$) in the kidney of trout exposed to elevated waterborne-Cu concentrations; the lowest water concentration had no effect relative to the control whereas there was an increasing contribution with higher water concentrations. There was no effect ($p = 0.93$) of waterborne Cu on the concentration of Cu in gill tissue. There was a strong effect of dietary Cu ($p < 0.001$) and no effect of waterborne Cu ($p = 0.52$) on the Cu concentration in digesta. The majority of measurements of the Cu content of bone were below the detection limit; visual inspection of the results revealed no trends.

Trout exposed to the highest waterborne-Cu concentration had elevated Zn concentrations in liver ($p = 0.025$) and marginally decreased concentrations in kidney ($p = 0.045$), although Tukey's analysis showed no pairwise differences in kidney tissue. There was no effect of dietary Cu on either liver ($p = 0.97$) or kidney ($p = 0.86$) Zn concentrations (Table 3).

The relative contributions of diet and water to Cu concentrations in liver were calculated for fish on the high-Cu diet. At the lowest level of waterborne-Cu exposure (38 $\mu g \cdot L^{-1}$), 1% of the liver Cu was attributable to the water. As waterborne-Cu concentration increased, so did its contribution to liver Cu; levels of 15 and 37% were apparent at 62 and 127 $\mu g\ Cu \cdot L^{-1}$, respectively.

Both waterborne ($p < 0.00001$) and dietary ($p = 0.019$) preexposure to Cu significantly increased the tolerance of rainbow

trout to waterborne Cu, although waterborne preexposure appeared to have a much greater impact (Table 4). Tukey's analysis revealed that for both diets, fish exposed to the highest level of waterborne Cu were significantly more tolerant than the fish in all other treatments, which were not significantly different from each other. There was no significant interaction ($p = 0.06$) between dietary-Cu and waterborne-Cu concentration with respect to Cu tolerance.

Discussion

We achieved our objective of inducing a relatively mild state of Cu toxicity in order to minimize the potential for direct effects of Cu toxicity on Cu metabolism and kinetics. Although chronic exposure to both dietary and waterborne Cu can result in depressed appetite, reduced growth, and altered food conversion efficiency (Lett et al. 1976; Collvin 1985; Lanno et al. 1985), no statistically significant impacts on these indicators were apparent.

The concentrations of Cu in liver, kidney, and gill increased as the Cu concentration in the diet increased. Several authors (Knox et al. 1982; Lanno et al. 1985; Gatlin et al. 1989) have noted elevated levels of hepatic Cu in fish exposed to dietary Cu. In situations of extremely high dietary Cu, rainbow trout will respond by sequestering Cu in distinct membrane-bound cytoplasmic inclusions in the hepatic parenchyma (Lanno et al. 1987). These inclusions could be a protective mechanism, akin to metallothionein synthesis, that protects cellular components from the toxic effects of the free Cu ion. These inclusions, have not, to our knowledge, been reported during waterborne-Cu exposure.

Consistent with previous reports (McCarter and Roch 1984; Lauren and McDonald 1987), Cu levels in liver and kidney increased with increasing waterborne-Cu concentration. Interestingly, Cu levels in gill increased with increasing dietary-Cu concentration but not with increasing waterborne-Cu concentration. Benoit (1975) reported significant increases in the concentration of Cu in the gills of bluegill (*Lepomis macrochirus*) exposed to 40 and 77 μg Cu·L^{-1}. In the present study, the strong effect of the high dietary-Cu concentration on the gill may have masked more subtle changes due to waterborne Cu.

Diet appeared to be the dominant source of Cu to rainbow trout tissues (particularly liver) during this study, although waterborne Cu became increasingly important as its concentration increased. Waterborne Cu accounted for up to 37% of the increased liver burden of fish on elevated Cu diets, despite the fact that dietary-Cu concentration was more than three orders of magnitude higher (680 versus 120 μg·L^{-1}). Given the large difference in concentration between the two routes of exposure, and the high efficiency of uptake of the respiratory route, we suspect that as the waterborne concentration increased, it would take over as the primary source. The results also suggest that, relative to respiratory uptake, Cu uptake from the diet is a well-regulated metabolic process.

Spry et al. (1988) measured whole-body Zn uptake in rainbow trout exposed simultaneously to both dietary (1, 90, or 590 μg·g^{-1}) and waterborne (7, 39, 148, or 529 μg·L^{-1}) Zn. As dietary-Zn concentration increased, trout retained, on a percentage basis, a decreasing proportion of the available Zn; at 590 μg Zn·g^{-1}, diet retention was only 2%. At elevated waterborne-Zn concentrations, however, Zn was taken up from the water regardless of the dietary load. Waterborne Zn represented up to 57% of the increased burden on Zn-adequate diets, even though the dietary-Zn concentration was three orders of magnitude higher.

Results similar to the above, which attribute the majority of tissue metals to waterborne and not dietary exposure, have been reported for both Se and Co. Bertram and Brooks (1986) investigated the relationship between Se concentrations in water and/or food and the kinetics of uptake, accumulation, and depuration of Se in fathead minnow (*Pimephales promelas*). The differences in uptake kinetics between Se in water and food suggested the existence of two functional compartments for the accumulation. Within 4 wk, the fish exposed to Se in water reached an equilibrium body burden of Se at a concentration 25.3 times that in water. Fish exposed to Se in food had not reached an equilibrium after 11 wk, but equilibrium concentrations were estimated (using a kinetics model) to be 0.3 times that in the food. These results compare favourably with other studies dealing with Se accumulation (Adams 1976; Hodson et al. 1980). Baudin and Fritsch (1989) investigated the relative contributions of food and water to the accumulation of Co by the common carp (*Cyprinus carpio*). Again, the data clearly indicated that water was the primary source of Co and that accumulation from both water and food was additive. Baudin and Fritsch (1987) suggested that this additive uptake was indicative of two separate kinetic compartments for Co assimilation, one driven by dietary Co and one by waterborne Co. In the present study, the accumulation of Cu in both the liver and the kidney appeared to be additive.

In our work, the most compelling support for a functional separation of Cu assimilated from water and diet comes from the Cu-tolerance levels of the fish at the end of the 42-d uptake study. Waterborne preexposure resulted, on the one hand, in a highly significant increase in Cu tolerance, consistent with previous reports (Dixon and Sprague 1981; Buckley et al. 1982; Roch and McCarter 1984). On the other hand, dietary preexposure resulted in only a marginally significant increase in Cu tolerance, although the liver-Cu levels in the fish receiving a high-Cu diet were more than double those receiving the low-Cu diet when both were exposed to comparable levels of waterborne Cu. The lack of a significant interaction between dietary-Cu and waterborne-Cu preexposure on Cu tolerance, although marginal, also suggests that the two routes were acting on tolerance independently.

Increased tolerance after waterborne preexposure to metals, including Cu, Zn, and Cd, has been associated with the induction of metallothioneins, particularly in liver (Roch and McCarter 1984; Hobson and Birge 1989; Olsson et al. 1989). Hepatic metallothioneins are thought to reduce metal toxicity and increase tolerance by sequestering metal ions away from metabolically important enzyme systems (Stegeman et al. 1992). Since dietary preexposure did not increase tolerance to the same degree as waterborne preexposure, it is also possible that metallothionein synthesis was not induced to the same degree. Although no direct information on metallothionein synthesis during dietary-Cu exposure is available, there is indirect support for this hypothesis from work with Zn. Whereas both waterborne (Bradley et al. 1985) and intraperitoneally injected (Overnell et al. 1988a) Zn elevates hepatic metallothionein levels in teleosts, dietary exposure does not (Overnell et al. 1988b).

The results reported here do not fully corroborate our previous field study (Miller et al. 1992) on the availability of Cu and Zn to white sucker. That study indicated that relative to dietary factors, aqueous metal concentrations were consistently a better predictor of tissue-metal levels. There are three potential

explanations for this, each or all of which could be contributing to the discrepancy. It is possible that the physiology of metal kinetics in white sucker is substantially different from that in rainbow trout. Alternatively, whereas the ratio of digesta-Cu (milligrams per kilogram) to waterborne-Cu (micrograms per litre) levels in the field situations never exceeded 8:1, they ranged as high as 26:1 in this study, perhaps facilitating Cu uptake from the gut. Finally, Cu may not be as available from the natural diets of the field study as it is from the practical diets that we used in the laboratory. Up to 60% of the metals in a natural diet composed largely of benthic invertebrates can be tightly bound to the cuticular proteins of the exoskeletons of those invertebrates (Milner 1979, 1982; Dallinger and Kautzky 1985) and hence are hypothesized to be unavailable for uptake.

In summary, simultaneous exposure of rainbow trout to dietary and waterborne Cu resulted in significant Cu assimilation. Diet proved to be the primary source of tissue Cu, although as waterborne-Cu concentration rose, its contribution increased. Cu from the two sources appeared to partition into functionally independent compartments; waterborne exposure was more effective at inducing Cu tolerance than dietary supplementation and did so at much lower levels of liver-Cu concentration. From a regulatory perspective, this work raises some concern regarding the role of fish tissue-metal levels in interpreting environmental impacts. Several authors (Connolly 1985; McGeachy and Dixon 1992) have suggested that the utility of water quality guidelines, which are currently based solely on waterborne-toxicant concentration, could be significantly increased by relating response thresholds to toxicant concentrations within organisms. This would account for contaminants that were available by routes other than direct aqueous exposure, such as diet. However, if contaminants taken up from water and diet are partitioned into compartments that are functionally different in terms of kinetics and thresholds of biological response, interpretation of the response associated with a particular tissue concentration will be more complex than originally anticipated.

Acknowledgements

Financial support for this work was provided by GECO Division, Noranda Minerals, Manitouwadge, Ont., and by an operating grant from the Natural Sciences and Engineering Research Council of Canada (A8155) to D.G.D. Statistical and technical assistance was provided by J. O'Hara-Hines and J. Waite, respectively.

References

ADAMS, W.J. 1976. The toxicity and residue dynamics of selenium in fish and aquatic invertebrates. Ph.D. thesis, Michigan State University, East Lansing, Mich. 184 p.

APHA. 1980. Standard methods for the examination of water and wastewater. 15th ed. American Public Health Association, Washington, D.C. 1334 p.

BAUDIN, J.P., AND A.F. FRITSCH. 1989. Relative contributions of food and water in the accumulation of ^{60}Co by a freshwater fish. Water Res. 23: 817–823.

BENOIT, D.A. 1975. Chronic effects of copper on survival, growth and reproduction of the bluegill (*Lepomis macrochirus*). Trans. Am. Fish. Soc. 104: 353–358.

BERTRAM, P.E., AND A.S. BROOKS. 1986. Kinetics of accumulation of selenium from food and water by fathead minnows. Water Res. 20: 877–884.

BRADLEY, R., W. DUQUESNAY, AND J.B. SPRAGUE. 1985. Acclimation of rainbow trout *Salmo gairdneri* Richardson to zinc: kinetics and mechanism of enhanced tolerance induction. J. Fish Biol. 27: 367–379.

BUCKLEY, J.A., M. ROCH, J.M. MCCARTER, C.A. RENDELL, AND A.T. MATHESON. 1982. Chronic exposure of coho salmon to sublethal concentrations of copper. I. Effect on growth, on accumulation and distribution of copper, and on copper tolerance. Comp. Biochem. Physiol. 72C: 15–19.

CCREM. 1987. Canadian water quality guidelines. Task Force on Water Quality Guidelines, Canadian Council of Resource and Environment Ministers, Environment Canada, Ottawa, Ont.

COLLVIN, L. 1985. The effect of copper on growth, food consumption and food conversion of perch *Perca fluviatilis* L. offered maximal food rations. Aquat. Toxicol. 6: 105–113.

CONNOLLY, J.P. 1985. Predicting single-species toxicity in natural water systems. Environ. Toxicol. Chem. 4: 572–582.

COUSINS, R.J. 1985. Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65: 238–309.

DALLINGER, R., AND H. KAUTZKY. 1985. The importance of contaminated food for the uptake of heavy metals by rainbow trout (*Salmo gairdneri*): a field study. Oecologia (Berl.) 67: 82–89.

DIXON, D.G., AND J.B. SPRAGUE. 1981. Acclimation to copper by rainbow trout (*Salmo gairdneri*) — a modifying factor in toxicity. Can. J. Fish. Aquat. Sci. 38: 880–888.

GATLIN, D.M. III, H.F. PHILLIPS, AND E.L. TORRANS. 1989. Effects of various levels of dietary copper and zinc on channel catfish. Aquaculture 76: 127–134.

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11: 714–719.

HOBSON, J.F., AND W.J. BIRGE. 1989. Acclimation-induced changes in toxicity and induction of metallothionein-like proteins in the fathead minnow following sublethal exposure to zinc. Environ. Toxicol. Chem. 8: 157–169.

HODSON, P.V., B.R. BLUNT, AND D.J. SPRY. 1978. Chronic toxicity of waterborne and dietary lead to rainbow trout (*Salmo gairdneri*) in Lake Ontario water. Water Res. 12: 869–878.

HODSON, P.V., D.J. SPRY, AND B.R. BLUNT. 1980. Effects on rainbow trout (*Salmo gairdneri*) of a chronic exposure to waterborne selenium. Can. J. Fish. Aquat. Sci. 37: 233–240.

HOLCOMBE, G.W., D.A. BENOIT, AND E.N. LEONARD. 1979. Long-term effects of zinc exposures on brook trout (*Salvelinus fontinalis*). Trans. Am. Fish. Soc. 108: 76–87.

KEVAN, S.D., AND D.G. DIXON. 1991. The acute toxicity of pulse-dosed thiocyanate (as KSCN and NaSCN) to rainbow trout (*Oncorhynchus mykiss*) eggs before and after water hardening. Aquat. Toxicol. 19: 113–122.

KNOX, D., C.B. COWEY, AND J.W. ADRON. 1982. Effects of dietary copper and copper:zinc ratio on rainbow trout *Salmo gairdneri*. Aquaculture 27: 111–119.

KNOX, D., C.B. COWEY, AND J.W. ADRON. 1984. Effects of dietary zinc intake upon copper metabolism in rainbow trout *Salmo gairdneri*. Aquaculture 40: 199–207.

LANNO, R.P., B. HICKS, AND J.W. HILTON. 1987. Histological observations on intrahepatocytic copper-containing granules in rainbow trout reared on diets containing elevated levels of copper. Aquat. Toxicol. 10: 251–263.

LANNO, R.P., S.J. SLINGER, AND J.W. HILTON. 1985. Maximum tolerable and toxicity levels of dietary copper in rainbow trout (*Salmo gairdneri* Richardson). Aquaculture 49: 257–268.

LAUREN, D.J., AND D.G. MCDONALD. 1987. Acclimation to copper by rainbow trout, *Salmo gairdneri*: biochemistry. Can. J. Fish. Aquat. Sci. 44: 105–111.

LEDUC, G. 1966. Une bouteille à débit constant pour petits volumes de liquides. Nat. Can. (Qué.) 93: 61–64.

LETT, P.F., G.L. FARMER, AND F.W.H. BEAMISH. 1976. Effect of copper on some aspects of the bioenergetics of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 33: 1335–1342.

LUOMA, S.N. 1977. Detection of trace contaminant effects in aquatic ecosystems. J. Fish. Res. Board Can. 34: 436–439.

MCCARTER, J.A., AND M. ROCH. 1984. Chronic exposure of coho salmon to sublethal concentrations of copper. III. Kinetics of metabolism of metallothionein. Comp. Biochem. Physiol. 77C: 83–87.

MCGEACHY, S.M., AND D.G. DIXON. 1992. Whole-body arsenic concentrations in rainbow trout during acute exposure to arsenate. Ecotoxicol. Environ. Saf. 24: 301–308.

MILLER, P.A., D.R. MUNKITTRICK, AND D.G. DIXON. 1992. Relationship between concentrations of copper and zinc in water, sediment, benthic invertebrates, and tissues of white sucker (*Catostomus commersoni*) at metal contaminated sites. Can. J. Fish. Aquat. Sci. 49: 978–984.

MILNER, N.J. 1979. Zinc concentrations in juvenile flatfish. J. Mar. Biol. Assoc. U.K. 59: 761–775.

MILNER, N.J. 1982. The accumulation of zinc by O-group plaice, *Pleuronectes platessa* (L.), from high concentrations in sea water and food. J. Fish Biol. 21: 325–336.

OLSSON, P.-E., A. LARSSON, A. MAAGE, C. HAUX, K. BONHAM, M. ZAFARULLAH, AND L. GEDAMU. 1989. Induction of metallothionein synthesis in rainbow trout (*Salmo gairdneri*) during long-term exposure to waterborne cadmium.

Fish. Physiol. Biochem. 6: 221–229.

OVERNELL, J., T.C. FLETCHER, AND R. MCINTOSH. 1988a. Factors affecting hepatic metallothionein levels in marine flatfish. Mar. Environ. Res. 24: 155–158.

OVERNELL, J., T.C. FLETCHER, AND R. MCINTOSH. 1988b. The apparent lack of effect of supplementary dietary zinc on zinc metabolism and metallothionein concentrations in the turbot, *Scophthalmus maximum* (Linnaeus). J. Fish Biol. 33: 563–570.

PART, P., AND O. SVANBERG. 1981. Uptake of cadmium in perfused rainbow trout (*Salmo gairdneri*) gills. Can. J. Fish. Aquat. Sci. 38: 917–923.

ROCH, M., AND J.A. MCCARTER. 1984. Hepatic metallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*) — I. Exposed to an artificial mixture of zinc, copper and cadmium. Comp. Biochem. Physiol. 77C: 71–75.

SINLEY, J.R., J.P. GOETTL, AND P.H. DAVIES. 1974. The effects of zinc on rainbow trout (*Salmo gairdneri*) in hard and soft water. Bull. Environ. Contam. Toxicol. 12: 192–201.

SPRY, D.J., P.V. HODSON, AND C.M. WOOD. 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45: 32–41.

STEGEMAN, J.J., M. BROUDER, R.T. DIGIULIO, L. FORLIN, B.A. FOWLER, B.M. SANDERS, AND P.A. VAN VELD. 1992. Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effects, p. 235–335. *In* R.J. Huggett, R.A. Kimerle, P.M. Mehrle Jr., and H.L. Bergman [ed.] Biomarkers: biochemical, physiological and histological markers of anthropogenic stress. Lewis Publishers, Chelsea, Mich.

Forecasting Abundance from Habitat Measures Using Nonparametric Density Estimation Methods

J.C. Rice

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada

Rice, J.C. 1993. Forecasting abundance from habitat measures using nonparametric density estimation methods. Can. J. Fish. Aquat. Sci. 50: 1690–1698.

The relationships between abundance of animals and characteristics of habitats are important for theoretical and management reasons. However, data on populations and habitats frequently are unsuitable for model-based statistical analyses. The shape of the functional relation between abundance and habitat and the form of the error distribution are rarely known accurately, and noise in the data often is large relative to the signal. Kernel estimators, a type of nonparametric probability density estimation technique, can use available data to estimate the probability density function (pdf) of abundance, given specified habitat conditions. One nonparametric estimator, related to kernel estimators and based on the Cauchy distribution, is described in detail. It is applied to data from three studies of influences of habitat on populations: stream characteristics on salmon and trout biomasses, depth of the 2°C isotherm on codtrap catches, and bottom temperature on capelin densities. Despite the differences among the data sets, the Cauchy kernel could be applied without modification in all cases. The pdf's of abundance were interpretable readily, captured dominant features of the biology of each species, and were suitable for management applications and for tests of hypotheses.

Les relations entre l'abondance des animaux et les caractéristiques des habitats sont importantes, pour des motifs théoriques et des motifs d'aménagement. Toutefois, les données que l'on possède sur les effectifs et sur les habitats conviennent rarement aux analyses statistiques fondées sur des modèles. La forme de la relation fonctionnelle entre l'abondance et l'habitat ainsi que la forme de la distribution de l'erreur sont rarement connues avec pré cision, et, dans les données, le rapport du bruit au signal est souvent important. Technique d'estimation non paramétrique de la densité de probabilité, les estimateurs de noyaux peuvent servir, à l'aide des données disponibles, à estimer la fonction de densité de probabilité (fdp) de l'abondance, dans des conditions données de l'habitat. On décrit dans le détail un estimateur non paramétrique, apparenté aux estimateurs de noyaux et fondé sur la loi de Cauchy. On l'applique aux résultats de trois études de l'influence de l'habitat sur les effectifs : les caractéristiques des cours d'eau sur la biomasse du saumon et de la truite, la profondeur de l'isotherme de 2°C sur les captures au moyen de trappes à morues et la température du fond sur la densité du capelan. En dépit des différences entre les ensembles de données, le noyau de Cauchy pourrait s'appliquer tel quel à tous les cas. Les fdp de l'abondance ont été faciles à interpréter. Ayant permis de saisir les caractéristiques dominantes de chaque espèce, elles étaient applicables aux problèmes d'aménagement ainsi qu'à la vérification des hypothèses.

Received December 23, 1991
Accepted March 8, 1993
(JB346)

Reçu le 23 décembre 1991
Accepté le 8 mars 1993

Ecologists have tried to relate the abundances of animal populations to attributes of habitat, particularly for studies of birds and mammals (e.g. Capen 1981; Ralph and Scott 1981; Terrell 1984; Verner et al. 1986). Interest in predicting abundances of animals from characteristics of their habitats is motivated both by theoretical ecology (MacArthur et al. 1966; Fretwell 1972; Cody 1974; Tilman 1982; MacNally 1983; Schoener 1983) and by needs of managers of fish and wildlife (Division of Ecological Services 1980; Department of Fisheries and Oceans 1986; Gatz et al. 1987; Smith et al. 1991).

Diverse multivariate techniques have been used to investigate how habitat is related to abundance, including several methods of ordination and canonical analysis and univariate and multivariate linear, curvilinear, and logistic regressions. A thorough and critical review by James and McCulloch (1990) underscores the shortcomings of all those methods to capture the relationships of abundance to habitat in statistically appropriate ways. Some of the problems identified in the review arise from "cavalier applications and interpretations" (James and McCulloch 1990, p. 159), for which there is little protection except rigorous peer review. However, many problems arise from inherent unsuitabilities of many common statistical methods for the data sampled from animal populations and habitats.

The statistical methods that James and McCulloch (1990) reviewed assume that relationships are smooth, continuous, and either linear or simple polynomials. They assume that error is drawn from simple, known distributions that are homogeneous among habitat attributes and across the observed range of abundances. Even after transformation, habitat and abundance data often fail to meet the necessary assumptions (Austin 1976; Williams 1981, 1983; Gotfryd and Hansell 1985). Applications sometimes forecast confidence intervals for abundance. However, accurate estimation of confidence intervals requires even stronger adherence to assumptions about distributions of errors and forms of functional relations than is necessary to estimate the mean. Moreover, the confidence intervals apply to parameters of models and of reference data sets, but not formally to predictions from them.

Notwithstanding these statistical problems, the past two decades of work on quantifying relationships between abundance and habitat have been productive. However, independent tests of quantified functional relations show at best weak predictive ability, and weak ability to test ecological hypotheses (Wiens 1985; Maurer 1986; Rotenberry 1986; Gatz et al. 1987; Morrison et al. 1987). The low but often statistically significant level of predictability may occur because the true associations actually are weak, or because predictive models only weakly capture the true associations between habitat characteristics and animal abundance.

Although ecological theories can yield predictions of how animals should use habitats (e.g., Fretwell 1972; May and MacArthur 1972; Dueser and Shugart 1979; MacNally and Doolan 1986), theory predicts in only general ways the shape of specific abundance–habitat functions. When abundance and habitat data are plotted, the relationships commonly show combinations of thresholds, floor and ceiling effects, asymmetric ascending and descending limbs, marked skewness or kurtosis, differing variability in abundance at different positions along a habitat gradient, and diverse other statistical problems (e.g., Rice 1978; Rice et al. 1981, 1986; Mannon and Meslow 1984). Curvilinear models may fit the data better than linear models, but they do not necessarily fit the data well (Meents et al. 1983; Rice et al. 1986).

Were theory to specify the shape of the abundance–habitat function, the relationship would have large errors because the abundance of animals at specific sites and times varies with many things. Substantial replicate sampling is necessary to obtain stable abundance estimates (Dawson 1981; Gates 1981; Wiens 1981; Johnson 1989) or to quantify habitat characteristics (Gotfryd and Hansell 1985). Correspondingly, which variables are included in abundance–habitat functions and the values of parameter estimates are both sensitive to the data selected for analyses and unstable under subsampling studies (Williams 1983; Gotfryd and Hansell 1985; Maurer 1986; Williams and Titus 1988).

In light of the above considerations, abundance at a site may be described better by a probability density function (pdf) than by a specific value and associated variances. Individual abundance estimates are then random samples from the pdf. Although replicate samples from the pdf may be variable, the properties of the pdf may be stable over spaces and times long enough to be of theoretical or management interest. Viewed in this context, samples from populations and habitats should be used to estimate the shape of the pdf as directly as possible, rather than to identify a single unknown functional relationship, and estimate the parameters of the function and its error term(s).

The family of probability density estimation methods are appropriate statistical tools to estimate pdfs. The statistical properties of these methods are well known (Tapia and Thompson 1978; Diggle 1985; Yakowitz 1985; Hastie and Tibshirani 1986; Silverman 1986). Although applications in ecology are infrequent (but see Rice and Evans 1988; Worton 1989), they are used widely in forecasting studies (Noakes et al. 1988; Noakes 1989). Simulation studies have shown that they perform as well or better (most often the smallest errors; least chance of being seriously in error) than model-based methods for prediction, unless the true model is specified accurately and variance in the data is small relative to the signal from the function (Yakowitz and Szidarovsky 1985; Evans and Rice 1988). Neither of those conditions is likely to be met for most studies quantifying the relationship of animal abundance to habitat.

In this paper, I describe a modified member of the class of nonparametric probability density estimation methods known as kernel estimators. I then illustrate its application to three studies of how habitat attributes influence abundance. The three examples were chosen from previously published fisheries studies to show the generality of the method with very different kinds of habitat attributes and abundance measures and the consistent ease of interpretation of the results.

Methods

Quantitative Methods

Rationale

The theory and mathematical formalizations of kernel estimators are developed in Silverman (1986). The kernel estimators described in Silverman (1986) estimate the pdf of expected values jointly in two random variables, by the average of the sum of kernels in the two variables. The type of estimators discussed in Diggle and Gratton (1984) address a related problem; they estimate the full pdf of one variable at a specific point on another, given a set of identically distributed reference data.

The task addressed by fisheries and habitat managers lies between those two problems. We have seen in the introduction that abundance data often are not identically distributed across the range of a habitat attribute. Nonetheless, managers commonly must estimate and advise on not just the most likely (expected) value or event, but on risks and ranges of possible outcomes under a specific set of conditions.

Evans and Rice (1988) developed a modified kernel estimator for the similar problem of estimating the pdf of recruitment for a specific spawning biomass. The full mathematical theory of their class of estimators has not been developed. However, using simulations, they explored the performance of their estimator relative to other nonparametric and model-based estimators for the class of problems requiring the full pdf at a specific value of another variable. Their modified method was superior (best results most often; least risk of going seriously wrong) to the alternatives tested. Application of their method to management problems was straightforward and provided useful results (Rice and Evans 1988). However, they cautioned that if a well-motivated specific model exists, or if reference data are known to be identically distributed, alternative methods with more demanding assumptions should be used. Also, there are cases when the estimates of model parameters are of inherent interest, rather than intermediate steps to a prediction.

The purpose of this paper is to illustrate application of this approach to habitat problems. For clarity, examples will be univariate, although multivariate extrapolations of kernel estimators have been developed (e.g., Loftsgaarden and Quesenberry 1965). The multivariate methods are computationally demanding, but conceptually are straightforward extensions of the univariate algorithms. Because examples are developed univariately, applications with several habitat attributes use an approach comparable to stepwise regression. This is for clarity of presentation and is not an endorsement of stepwise methods (James and McCulloch 1990). The approach does bring out the parallel with nonparametric regression, which is an alternative to density estimation when one expects the relationship to be smooth and continuous and the variance in abundance to be consistent across the range of the habitat attribute.

Algorithms

If the abundance of an animal at a site of interest ("test site") is considered to be a pdf, then the problem is: Given a set of observations of abundances and habitat attributes from past sampling ("reference data" from "reference sites"), and measures of the habitat attributes at the test site, estimate the corresponding abundance pdf. Our modified kernel method estimates the pdf by weighting each observed abundance in the reference data set according to the similarity of the habitat attribute(s) of that reference site to the habitat attributes(s) at the test site.

For comparison, were the reference data used in a regression model, data from all reference sites would be used to estimate the slope(s) and intercept of the regression equation. The leverage of each reference site (i.e., the relative influence of each observation on the slope and intercept) depends on the closeness of each observation to the mean(s) of the independent variable(s). However, a given reference site would have equal influence in the prediction of any test site, regardless of similarity of the test site to the reference site.

The kernel estimator rigorously quantifies "similarity" and schedules how influence falls off with decreasing similarity. Generally, the choice of the exact kernel algorithm has much less influence on the pdf produced than does the value(s) of the parameter(s) of the kernel (Wegman 1972; Bowman 1985; Gasser et al. 1985; Silverman 1986). We use a kernel based on the Cauchy distribution, rather than the commonly used Normal kernel, because a Normal kernel may give very different weights to observations close together but distant from the test site. The Cauchy algorithm used is

$$(1) \qquad W_i = \frac{1}{1 + \left(\frac{X_i}{D}\right)^2}$$

where W_i = weight of the ith observation, X_i = distance between value of the habitat attribute at the ith reference site and the test site, and D = the "tuning" parameter, in the same units as X_i. When X_i is small relative to D (sites are similar), W_i is large; when X_i is large relative to D (sites differ greatly), W_i is small. When D is large relative to all X_i, all weights converge to 1, so all points receive equal weight. This represents the null hypothesis that one cannot forecast the pdf of abundance from the habitat attribute, so the best prediction of a pdf of abundance is the unweighted frequency distribution of observations.

Among the reasons for using nonparametric density estimation methods instead of parametric models are lack of knowledge of the true distributions of errors and lack of smoothness in the unknown true functional relations. Therefore, to measure goodness of fit the deviations of observations from the pdf median may be more appropriate than deviations from the pdf mean. The sum of squared deviations from the median is then the least-squares criterion to be minimized, but it is not a true variance.

The optimum value of D is chosen through cross-validation (Efron and Gong 1983; Bowman et al. 1984; Hall and Marron 1990). Begin with a trial D much larger than the range of values of the habitat observations. Estimate the cross-validated sum of squares value. (That is, delete one observation in the reference data set. Use equation 1 to estimate the pdf for that observation using the value of the habitat attribute at the deleted site and all other pairs of observations in the reference data set. Calculate the squared difference of the abundance at the deleted site from the median of the estimated pdf of abundance. Repeat for each observation in the reference data set and sum the squared differences.) This cross-validated sum of squares measures goodness of fit, assuming the null hypothesis of no relationship. For progressively smaller trial D, repeat the estimation of cross-validated sum of squares. The optimal value of D from a reference data set is the value that minimizes the cross-validated sum of squares. It should not be too large relative to the range of the reference habitat data, in order to ensure tolerably small bias in the estimated pdf (Silverman 1986).

In the applications presented here, weights of observations are accumulated as cumulative pdf's, or ogives, which are scaled to 1. No further smoothing is performed on the step-function ogives. The step-function ogives make it easier for readers to see the relationship between the individual observations in the reference data sets and the predicted ogive. In real applications, further smoothing of the ogives may be desirable (e.g., Diggle 1985) or steps can be binned and presented as histograms. When smoothed, the cumulative ogives are continuous and can be illustrated as probability distributions.

A prediction for a test site is a full pfd, not a single expected value. Therefore, plots will be probability (y-axis) versus abundance (x-axis) for a given value of the habitat attribute, not the usual regression-based plot of expected value of abundance across the range of the habitat variable. Hence, the full uncertainty of each prediction is an intrinsic part of the results. The uses of the nonparametric methods can be seen best when compared with conventional methods for analysis of the same data. For each example, regression analyses also are presented. For the salmon, trout, and cod data, the regressions duplicated analyses reported in publications. For the capelin example, linear and quadratic regressions of both raw and log-transformed abundance data were conducted, and the results with highest r^2 were selected. The analyses are to be taken as conventional benchmarks, and not necessarily definitive. For consistency, all regression analyses were done using PROC REG of SAS (SAS Institute Inc. 1982).

Data Sets

Atlantic salmon (Salmo salar) *and brook trout* (Salvelinus fontinalis), *Highland River, Newfoundland*

Highland River rises on the west coast of Newfoundland and flows into the Gulf of St. Lawrence. In a 3-yr study, Gibson et al. (1987) estimated standing stocks of salmon and trout by electrofishing at selected sites on the river. Sites were chosen to represent all major habitat types along the river. All fish sampled were aged by reading scales. Total biomass, biomass by age, and production at age were estimated from the sampling data. For the examples reported here, I used estimates of total biomass of salmon and total biomass of trout at each site as the estimates of abundance.

Project staff measured an extensive set of biotic and abiotic habitat attributes. Six were selected as likely candidates for evaluating habitat quality, on the basis of ease and consistency of measurement by field managers, and relevance to salmonid life histories. The attributes were mean width of stream, percent cover, height of ice scour mark, stream velocity, minimum stream depth, and substrate particle size. Preliminary analyses had indicated high intercorrelations among these six attributes. Cover, depth, and substrate grain captured almost 90% of the among site variation in all six measures. These three served as the habitat attributes in the examples. All details of sampling of

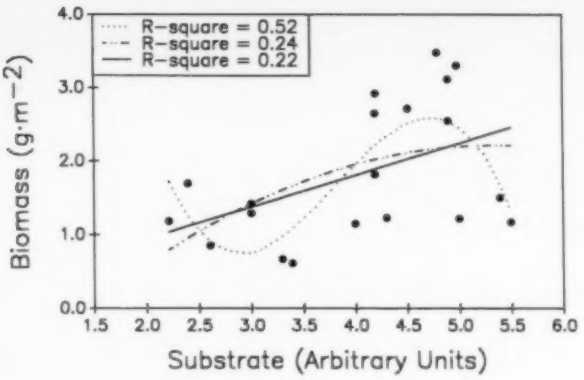


FIG. 1. Scatterplot of Atlantic salmon biomass and substrate grain (arbitrary scale) for samples collected in Highland River, Newfoundland. Lines are for linear (solid), quadratic (dashed – dotted), and third-power (dotted) polynomial regression models fit to the data. Were all assumptions of regression met by the data, all regression lines would be significant ($P < 0.05$).

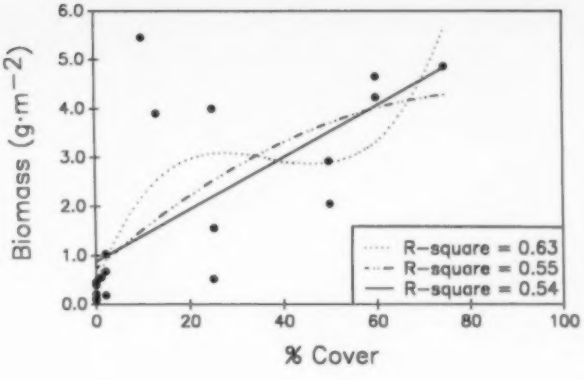


FIG. 2. Scatterplot of brook trout biomass and percent cover over stream for samples collected in Highland River, Newfoundland. Regression lines are as in Fig. 1. Were all assumptions of regression met by the data, all regression lines would be significant ($P < 0.05$).

salmonid populations and habitat attributes and preliminary analyses are reported in Gibson et al. (1987).

Trap catches of Atlantic cod (Gadus morhua), *Conception Bay, Newfoundland*

Atlantic cod in the waters of the Northeast Newfoundland Shelf and Labrador Sea typically migrate to nearshore areas during summer. The inshore concentrations support important fisheries prosecuted by several gears, including the traditional cod trap. Landings by this passive gear fluctuate substantially among communities within years, and among years at specific communities (Lear et al. 1986).

In 1986, the inshore codtrap fishery in Conception Bay, Newfoundland, was studied. Several traps were instrumented to record water temperature profiles. Daily catches in each trap and the associated temperature profiles were recorded. The contractor conducting this study had selected the depth of the 2°C isotherm as the relevant habitat attribute and catch in kilograms as the measure of abundance. Details of the field methods and justifications for selection of variables are reported in Aggett et al. (1987).

Density of capelin (Mallotus villosus), *southeast shoal of Grand Bank*

Capelin are a boreal pelagic species. They form dense aggregations during the spawning period, generally late spring and early summer. Most capelin stocks are beach spawners, but the southern Grand Bank stock spawns on top of the Southeast Shoal at depths from 50 to 100 m.

Since the early 1980's, hydroacoustic research surveys have been conducted on this capelin stock, during the period of aggregation on the Southeast Shoal. Following a detailed research sampling design, hydroacoustic estimates of capelin density are made for survey blocks, and bottom temperatures are recorded in each block. Integrated analyses of these data have not been completed, but ongoing work indicated that variation in bottom temperature influenced capelin distribution and, indirectly, capelin reproductive success. One of the impediments to comprehensive analyses of these relationships was the unsuitability of the abundance data for many types of statistics, due to the patchy, dense aggregations. References for the hydroacoustic survey methods and data for providing indices of capelin abundance are presented in Carscadden et al. (1989).

Results

Salmon and Trout

Regression analysis

The scatter plot of salmon biomass and substrate particle size shows substantial variability in biomass at moderate to large grain, and low salmon biomass at fine grains (Fig. 1). All polynomial regressions up to order 3 fail to reflect important aspects of the data. The biomass of trout is very low at open sites, highly variable at sites of low cover, and fairly high at all sites of moderate to high cover. Although the polynomial of order 3 reflects some of the pattern in the data (Fig. 2), this curve does not reflect the variance at low cover, and would be very dangerous if used to extrapolate to high cover levels. For both salmon and trout, relationships with other independent variables are weaker.

Kernel analysis: salmon

With a very large value of D, the cross-validation sum of squares of salmon biomass was 1.02. Of cover, substrate, and depth, the largest reduction in cross-validated sum of squares came when the pdf of abundance was forecast from substrate grain. The residual sum of squares was 0.56, for a reduction of 45%. This reduction came with a value of D between 0.36 to 0.38, around one eighth the observed range of substrate grain. Using this value of D, ogives are drawn for four hypothetical sites, with substrate grain covering the range of observed values. For substrate grain finer than 3.3, there is a high probability (>0.6) of a biomass below 1.2 and a low probability (<0.15) of a biomass above 1.7 (Fig. 3). For an intermediate grain size of 4.4, a wide range of biomasses are possible, although there is a greater than 50% chance of a biomass greater than 2.5. For even coarser substrates, intermediate biomasses are most likely, with only around a 20% chance of biomasses less than 1.2 or greater than 2.7. Note the gradual slope of the ogives at grains of 4.4 and 5.6, corresponding to the variability in the observations. The shallow slope indicates a large uncertainty in expected value for

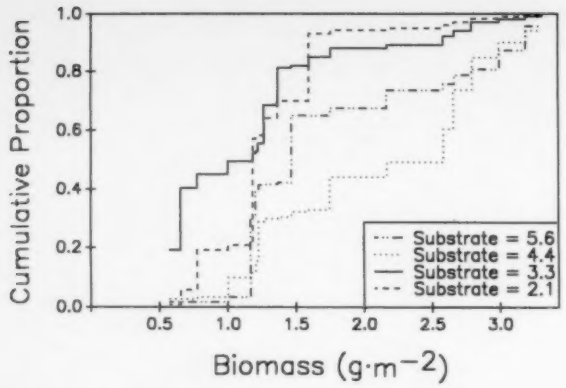


FIG. 3. Ogives of Atlantic salmon biomass for four trial substrate grains. Trial values were chosen to cover the observed range of substrate grain but not to coincide with any observation.

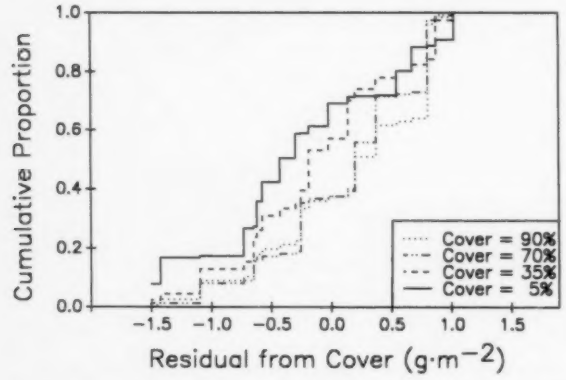


FIG. 4. Ogives of the residuals of Atlantic salmon biomass after accounting for the influence of substrate for four trial values of percent cover over stream. Residuals were calculated by estimating a cross-validated pdf based on substrate for each data point in Fig. 1 and subtracting the pdf median from the observed biomass at the site.

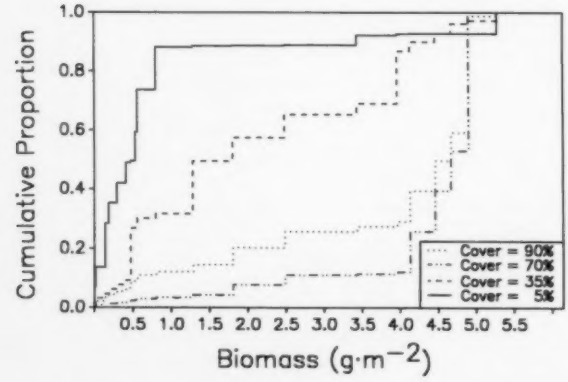


FIG. 5. Ogives of brook trout biomass for four trial levels of cover. Trial values were chosen to include the observed range of cover but not to coincide with any observation.

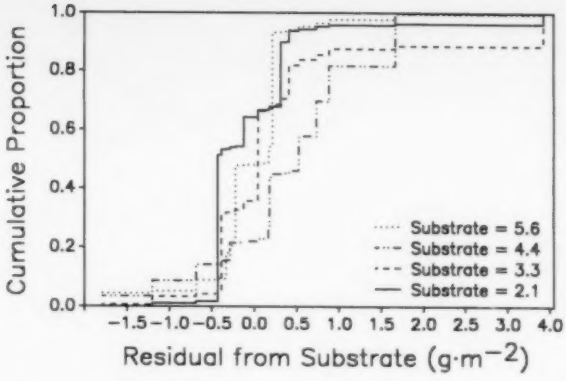


FIG. 6. Ogives of the residuals of brook trout biomass after accounting for the influence of cover for four trial values of substrate grain. Residuals were calculated as in Fig. 3 for cross-validated pdf's based on cover.

individual samples from such sites.

Once the effect of substrate grain on the pdf of abundance was estimated, residuals of observed values and pdf medians were calculated, and their relationships with depth and cover were explored. Depth accounted for none of the residual variability (no value of D produced a cross-validated sum of squares lower than with the largest D value), but cover accounted for 8% of the residuals in substrate. With a large value of D, cross-validated sum of squares of residuals was 0.61; with $D = 11.0$, sum of squares was 0.56. For test sites, all ogives are fairly shallow (Fig. 4). The ogives suggest that sites with very low cover (5%) have a fairly high probability (>70%) of negative residuals. For sites with a given substrate grain, ones with very low cover probably will have slightly lower abundances than expected from the type of substrate. From the shape of the ogives, predictions for open, fine-grained sites have the least uncertainty (i.e., narrowest range of likely values). Predictions for sites of intermediate grain and high cover will have a wide range of likely values.

Kernel analysis: trout

With large values of D, the cross-validated sum of squares in trout biomass was 5.76. The reduction in cross-validated sum of squares was greatest for cover; with D in the range 2.0–2.4, sum of squares was 1.66, for a reduction of over 70%. For a series of hypothetical sites the influence of cover on trout abundances is apparent. The ogive is very steep for 5% cover, with less than a 0.1 probability of a biomass greater than 1.0 (Fig. 5). For 35% cover, there is still a 20% chance of a very low bio mass, but the shallow and even slope between biomasses of 0.5 and 4 suggests that biomasses anywhere in this wide range have a similar likelihood of being observed. For cover of 70%, there is a very low likelihood of biomass less than 4.0. For very high cover, the ogive is still steep above 4.0, but there is nearly a 40% chance of observing biomasses anywhere in the wider range from 0.5 to 4.0.

Residuals from the reference data were calculated, given the observed cover values and the cross-validated optimum value of D. These residuals were again unrelated to depth, but 8.9% of the residual sum of squares was explained by pdf's based on substrate grain with $D = 0.22$ (Fig. 6). At fine (2.1 and 3.3) or coarse (5.6) substrates the ogives are steep between −0.5 and +0.3. This suggests that trout biomass is not strongly affected by

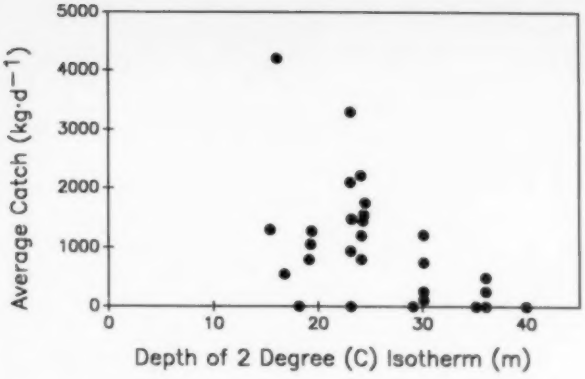


FIG. 7. Scatterplot of catch of Atlantic cod and depth of the 2°C isotherm for monitored cod traps in Conception Bay, Newfoundland, in the 1986 summer fishery.

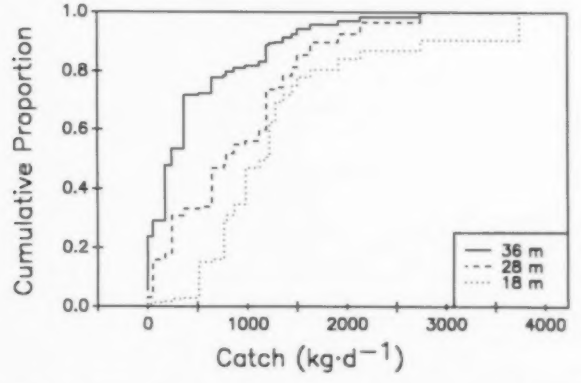


FIG. 8. Ogives of catch of Atlantic cod for three trial depths of the 2°C isotherm, based on data in Fig. 7. Trial values cover the range of water profiles observed in Conception Bay in 1986 but do not coincide with any data point in Fig. 7.

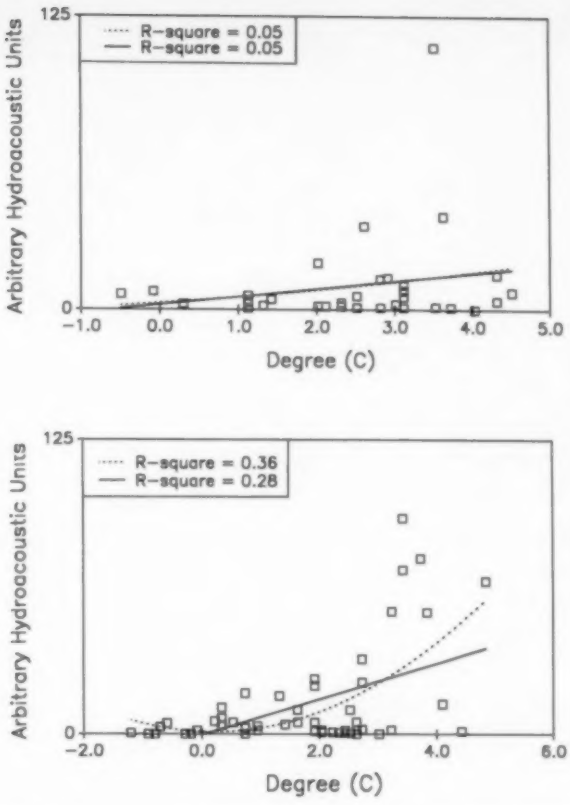


FIG. 9. Scatterplots of capelin density (arbitrary units from hydroacoustic records) and bottom temperatures for Southeast Shoal, Grand Bank, Northwest Atlantic Ocean, in 1981 (top) and 1987 (bottom). Linear (solid) and quadratic (dashed) regression lines are not significant in 1981 ($P > 0.05$). In 1987, the linear regression is significant, but the quadratic term does not explain significant additional variance. Densities presented in log scale to show range of observations.

extreme substrate levels, given a specific level of cover. For intermediate (4.4) grain, however, the ogive is somewhat less steep, with residuals likely to be from the range −0.4 to + 1.5. This suggests that for substrate of intermediate grain, trout biomass is likely to be somewhat higher than expected given the level of cover.

Codtrap Catches versus Water Temperature

Regression analysis

Aggett et al. (1987) noted the wide scatter and nonlinearity of the catch–temperature relationship. Their analyses fit quadratic models to the data. To allow parameters of quadratic regression models to be estimated, the authors constrained all polynomials to pass through (0,0). Without this constraint, quadratic terms were not significant. Using their constraint the top of each curve was just to the left of the first observation in the scatterplot (Fig. 7). This suggests that the (0,0) constraint, which lacked any biological justification, was largely responsible for the parameter estimates. Predictions of such models are unreliable. Moreover, the models do not represent the large uncertainty present in the observations, nor how the uncertainty changes along the habitat axis. Other covariates may be important, but were not recorded.

Kernel analysis

The cross-validated sum of squares in trap catch per day is 11.3×10^5 . kg^{-2}, with a D value of 100 (much greater than the observed range of depths, 16–40 m). A D value of 3.0 m reduces the sum of squares 28%. The overall fit was no better than the linear regression ($r^2 = 0.27$). However, the regression analysis produced an implausibly high intercept (2165 kg with the 2°C isotherm at the surface), predicted "expected" catches above 2000 kg whenever the 2°C isotherm was above 8 m, and the pattern of residuals was unacceptable. The performance of the kernel estimator is superior on these features.

Using the kernel estimators for the hypothetical case of the 2°C isotherm at 36 m, the ogive is very steep (Fig. 8). Trap catches under these very cold conditions have a probability less than 0.25 of exceeding even 400 kg. For somewhat warmer conditions, with the 2°C isotherm at 28 m, the ogive suggests that catches can be anywhere between 200 and 2000 kg, but are very unlikely to exceed 2000 kg. For the warmest test condition

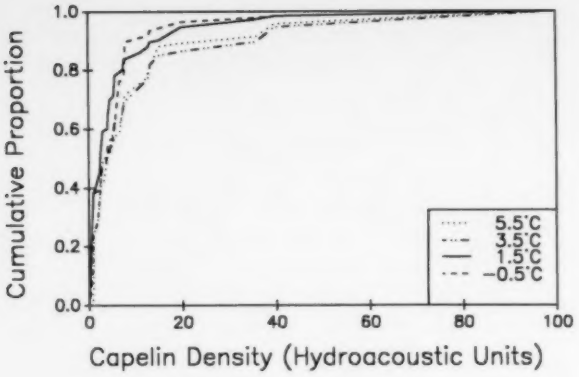


FIG. 10. Ogives of capelin density for four trial values of bottom temperature, based on the 1981 data in Fig. 9 (top).

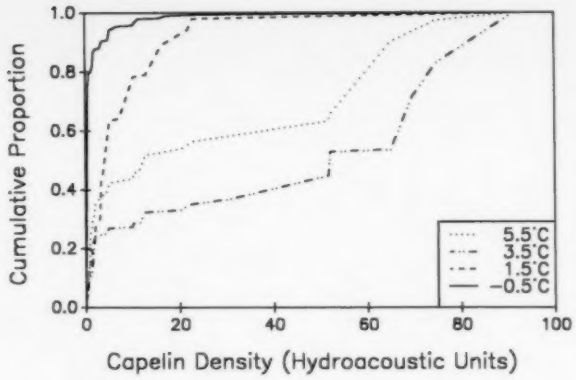


FIG. 11. Ogives of capelin density for four trial values of bottom temperature, based on the 1987 data in Fig. 9 (bottom)

(18 m), catches below 500 kg are very unlikely, and there is a 0.5 probability that catches exceed 1500 kg. The very good catches of 2000–4000 kg still have a probability of only about 0.15. That is the reality of the fishery. There is no temperature regime that ensures very good catches. To have more than a negligible chance at such catches, however, the 2°C isotherm had to be above 20 m.

Capelin Density versus Water Temperature

Regression results

Stepwise polynomial regression suggests that there is little relationship between density and temperature in 1981, and a modest curvilinear one in 1987. Note that the quadratic function underestimates abundances at temperature above 2°C and suggests that density is increasing at negative temperatures (Fig. 9).

Kernel analysis

The 1981 data have a cross-validated sum of squares of 4.2×10^2. No value of D reduces this sum of squares more than 1.5%. This suggests that the 1981 data do not permit forecasting of capelin density from temperature. The very steep left limb of the ogives (Fig. 10) at a variety of temperatures reflects the low density that prevailed at most sites. Log-transformations of density do not change these relationships.

In 1987, there were more cases of high densities of capelin. The total cross-validated sum of squares is correspondingly greater, 7.4×10^2. At a D value of 0.40 the cross-validated sum of squares is reduced 53% to 3.5×10^2. This is substantially better than the regression analyses of these data. The ogives at different test temperatures are markedly different as well (Fig. 11). For very cold water (–0.5°C) the ogive resembles the 1981 ogives, rising steeply with little likelihood of capelin densities above 10. A 2°C increase to +1.5°C changes the ogive very little. Another 2° increase to 3.50°C changes the ogive dramatically. There is still a 0.25 probability of very low density but a 0.50 probability of capelin density greater than 70 units. Again, water temperatures that appear preferred do not ensure that dense schools of capelin will be present.

For 1987, the nearly flat ogive between 2 and 70 reflects the schooling nature of capelin. In years when capelin are abundant, if they are present in an area, they are likely to be in dense schools. The ogives reflect this feature of capelin biology, estimating relatively high probabilities of either small or large densities, but low likelihood of intermediate values. The quadratic regression equation produces a line falling between the low and high densities, making an intermediate abundance the "expected" value even though it rarely occurs. The kernel estimation methods do not create relationships where none exist and handle data from aggregated species much more appropriately than regression models do.

Discussion

The three examples cover many of the problems of habitat and abundance data. The salmon and trout abundance data were sampled directly, with frequent low observations; features of the habitat were measured on several different scales. The cod trap catch data had a large trend in variance in abundance as depth of the 2°C isotherm rose, and catches were highly skewed. The capelin were strongly aggregated, with abundance either high or low. Despite these differences, the kernel estimator could be used without modification in each application.

The dominant features of the abundance data were preserved in even the unsmoothed predicted pdf's. This facilitates interpretation in the context of the biology of the population under study, and differs from many model-based analysis methods. There, predictions may be biased by distributional characteristics of the observations, but the predictions do not necessarily preserve those characteristics. Moreover, one or two outliers or arbitrary constraints (as in the published codtrap analysis) will affect parameter estimates of a regression strongly, and hence all predictions using the model. In the kernel methods, the outlier(s) will be present as an extended limb of the pdf, but the influence of the outlier(s) diminishes quickly outside the area of the habitat axis where the exceptional observations lie.

Although a few outliers do not introduce serious bias to all predictions from density estimation methods, bias is still a concern. Generally, large numbers of observations are needed before kernel estimators provide completely unbiased estimates of pdf's (Bowman 1985; Silverman 1986). However, Walters (1985) has shown that there is likely to be serious bias in parameter estimates for many model-based analyses of abundance data, even under circumstances where there is some theoretical motivation for the model used. Habitat managers are in a worse situation. They rarely have any a priori justification for assuming any particular form for the relationship. The pdf's forecast

by the kernel methods are likely to contain some bias, but they are likely to be wrong by less than predictions from routine model fitting to a few dozen noisy data points (Aitchison and Lauder 1985; Yakowitz 1985; Yakowitz and Szidarovsky 1985; Evans and Rice 1988).

The pdf's also contain much more information than is commonly taken from model-based analyses. In theory, model-based analyses can also produce complete pdf's. However, pdf's from model-based analyses are accurate only if under lying assumptions about the functional form and shape of the true error distribution are met. Because pdf's present more information to users, more interpretation is required of them. If users require only the expected abundance across the range of the habitat attribute, then the formal kernel methods described in Silverman (1986) are appropriate. However, the modified methods allow flexibility in selecting an "expected" value (median, mean, mode). They also allow direct estimation of probability intervals appropriate to a specific problem, e.g., the probability of a good catch under a specific environmental condition, or the probability that the population falls below some critical value if the habitat is in a particular state.

The information content of the pdf's tends to diminish emphasis on the overall level of significance of model parameters, although computational methods allow evaluation of significance of kernels (Efron 1981; Silverman 1986). For example, using the reference data set, pdfs can be bootstrapped to determine if the unweighted cumulative frequency distribution of abundance observations lies within the range of pdf's produced by the kernel estimators. For a pair of ogives from different values of the habitat attribute(s), differences in probabilities of the full range of possible outcomes can be tested, e.g., with simple Kolmogorov–Smirnov tests (Sokal and Rohlf 1973).

The information in the full pdf's can be used in many ways. When testing an a priori hypothesis, the slope of the pdf provides important information about the range of estimates consistent with a prediction. Possibly the low predictability in tests of habitat-based regression models (Maurer 1986; Rotenberry 1986; Morrison et al. 1987) arises from the large uncertainty that is not reflected in the model predictions, rather than from a failure of the models to capture imporant aspects of the relationship of abundance to habitat. The direct estimation of the probability that a population falls above or below some value given a specified set of conditions may be useful in risk assessment.

Providing pdf's to managers requires that they consider what properties of abundance are crucial to management. As with the codtrap data, there may be no conditions that ensure a large population. Nonetheless, desired population levels may be shown to be more probable in some habitat conditions than others. Managers may target for highest possible probability of large populations, lowest probability of small populations, or other specifiable targets. In each case, users will have more realistic expectations of the range of outcomes likely to be observed in studies of short duration, even if the habitat objectives have been met.

Many other extensions of the application of kernel estimators to abundance–habitat relationships are possible. If the habitat data include spatial coordinates, maps of pdf's of abundance can be constructed, as other methods of spatial analysis such as kriging can produce maps of abundance. In these applications, nonparametric density estimation methods again outperform conventional methods, for all but ideal cases (Yakowitz and Szidarovszky 1985). These maps are informative in themselves, and resampling of the pdf's can provide abundance estimates with associated variances that also do not depend on untenable assumptions.

This paper has shown that kernel estimators can be applied to diverse types of habitat and abundance data. Results are consistently direct and biologically interpretable. By making no assumptions about shapes of underlying relationships or error structures, they improve on current practice in many areas of weakness pointed out by James and McCulloch (1990).

Acknowledgments

I began this work while at the Northwest Atlantic Fisheries Centre, St. John's, Newfoundland. My appreciation of the value of density estimation methods developed through collaboration with Dr. Geoff Evans, whose intellectual rigour has been stimulating. John Gibson and Jim Carscadden generously provided research data for two of the examples, before they had completed their own publications. Don Noakes introduced me to a large body of forecasting literature that documents we had reinvented a quantitative wheel. Colin Levings, Don Noakes, and two anonymous reviewers provided valuable comments on this manuscript.

References

AGGETT, D., H.S. GASKILL, D. FINLAYSON, S. MAY, C. CAMPBELL, AND J. BOBBITT. 1987. A study of factors influencing availability of cod in Conception Bay, Newfoundland. *In* W.H. Lear and J.C. Rice (ed.) Can. Tech. Rep. Fish. Aquat. Sci. 1562.

AITCHISON, J., AND I.J. LAUDER. 1985. Kernel density estimation for compositional data. Appl. Stat. 34: 129–137.

AUSTIN, M.P. 1976. On non-linear species response models in ordination. Vegetatio 33: 33–41.

BOWMAN, A.W. 1985. A comparative study of some kernel-based nonparametric density estimators. J. Stat. Comput. Simul. 21: 313–327.

BOWMAN, A.W., P. HALL, AND D.M. TITTERINGTON. 1984. Crossvalidation in nonparametric estimation of probabilities and probability densities. Biometrika 71: 341–351.

CAPEN, D.E. (ed.) 1981. The use of multivariate statistics of studies of wildlife habitat. Rocky Mountain Forest and Range Experimental Station. U.S. For. Serv. Gen. Tech. Rep. RM-87. U.S. Department of Agriculture; Fort Collins, Colo. 249 p.

CARSCADDEN, J.E., K.T. FRANK, AND D.S. MILLER. 1989. Capelin (*Mallotus villosus*) spawning on the Southeast Shoal: influence of physical factors past and present. Can. J. Fish. Aquat. Sci. 46: 1743–1754.

CODY, M.L. 1974. Competition and the Structure of Bird Communities. Monogr. Popul. Biol. No. 7. Princeton University Press; Princeton, N.J. 318 p.

DAWSON, D.G. 1981. Experimental design when counting birds, p. 392–398. *In* C.J. Ralph and J.M. Scott (ed.) Estimating numbers of terrestrial birds. Stud. Avian Biol. No. 6. Allen Press, Lawrence, Kans.

DEPARTMENT OF FISHERIES AND OCEANS. 1986. The Department of Fisheries and Oceans policy for the management of fish habitat. Communications Directorate, Ottawa, Ont. Fs23-98/1986e. 38 p.

DIGGLE, P. 1985. A kernel method for smoothing point process data. Appl. Stat. 34: 138–147.

DIGGLE, P.J., AND R.J. GRATTON. 1984. Monte Carlo methods of inference for implicit statistical models. J. R. Stat. Soc. Br. 46: 193–227.

DIVISION OF ECOLOGICAL SERVICES. 1980. The habitat evaluation procedures. 102 ESM. U.S. Fish and Wildlife Service, Washington, D.C.

DUESER, R.D., AND H.H. SHUGART, JR. 1979. Niche pattern in a forest-floor small-mammal fauna. Ecology 60: 108–118.

EFRON, B. 1981. Nonparametric standard errors and confidence intervals. Can. J. Stat. 9: 139–172.

EFRON, B., AND G. GONG. 1983. A leisurely look at the bootstrap, jackknife and cross-validation. Am. Stat. 7: 1–26.

EVANS, G.T., AND J.C. RICE. 1988. Predicting recruitment from stock size without the mediation of a functional relation. J. Cons. Int. Explor. Mer 44: 111–122.

FRETWELL, S.D. 1972. Populations in a seasonal environment. Monogr. Popul. Biol. No. 5. Princeton University Press, Princeton, N.J. 217 p.

GASSER, T., H.-G. MULLER, AND V. MAMMITZSCH. 1985. Kernels for nonpara-

metric curve estimation. J. R. Stat. Soc. Br. 47: 299–315.

GATES, C.E. 1981. Optimizing sampling frequency and numbers of transects and stations, p. 399–404. *In* C.J. Ralph and J.M. Scott (ed.) Estimating numbers of terrestrial birds. Stud. in Avian Biol. No. 6. Allen Press, Lawrence, Kans.

GATZ, A.J., JR., M.J. SALE, AND J.M. LOAR. 1987. Habitat shifts in rainbow trout: competitive influences of brown trout. Oecologia 74: 7–19.

GIBSON, R.J., T.R. PORTER, AND K.G. HILLIER. 1987. Juvenile salmonid production in the Highlands River, St. George's Bay, Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. 1538.

GOTFRYD, A., AND R.I.C. HANSELL. 1985. The impact of observer bias on multivariate analyses of vegetation structure. Oikos 45: 223–234.

HALL, P., AND J.S. MARRON. 1990. On variance estimation in nonparametric regression. Biometrika 77: 415–419.

HASTIE, T., AND R. TIBSHIRANI. 1986. Generalized additive models. Stat. Sci. 1: 297–310.

JAMES, F.C., AND C.E. MCCULLOCH. 1990. Multivariate analysis in ecology and systematics: panacea or Pandora's box? Annu. Rev. Ecol. Syst. 21: 129–166.

JOHNSON, D.H. 1989. An empirical Bayes approach to analyzing recurring animal surveys. Ecology 70: 945–952.

LEAR, W.H., J.W. BAIRD, J.C. RICE, J.E. CARSCADDEN, G.R. LILLY, AND S.A. AKENHEAD. 1986. An examination of factors affecting catch in the inshore fishery of Labrador and Eastern Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. 1469.

LOFTSGAARDEN, D.O., AND C.P. QUESENBERRY. 1965. A non-parametric estimate of a multivariate density function. Ann. Math. Stat. 36: 1049–1051.

MACARTHUR, R.H., H. RECHER, AND M.L. CODY. 1966. On the relation between habitat selection and bird species diversity. Am. Nat. 100: 319–332.

MACNALLY, R.C. 1983. On assessing the significance of interspecific competition to guild structure. Ecology 64: 1646–1652.

MACNALLY, R.C., AND J.M. DOOLAN. 1986. An empirical approach to guild structure: habitat relationships in nine species of eastern-Australia cicadas. Oikos 47: 33–46.

MANNON, R.W., AND E.C. MESLOW. 1984. Bird populations and vegetation characteristics in managed and old-growth forests, northeastern Oregon. J. Wildl. Manage. 48: 1219–1238.

MAURER, B.A. 1986. Predicting habitat quality for grassland birds using density-habitat correlations. J. Wildl. Manage. 50: 556–566.

MAY, R.M., AND R.H. MACARTHUR. 1972. Niche overlap as a function of environmental variability. Proc. Natl. Acad. Sci. USA 69: 1109–1113.

MEENTS, J.K., J.C. RICE, B.W. ANDERSON, AND R.D. OHMART. 1983. Non-linear relationships between birds and vegetation. Ecology 64: 1022–1027.

MORRISON, M.L., I.C. TIMOSSI, AND K.A. WITH. 1987. Developing and testing of linear regression models predicting bird-habitat relationships. J. Wildl. Manage. 51: 247–253.

NOAKES, D.J. 1989. A nonparametric approach to generating inseason forecasts of salmon returns. Can. J. Fish. Aquat. Sci. 46: 2046–2055.

NOAKES, D.J., K.W. HIPEL, I.A. MCLEOD, C. JIMENEZ, AND S. YAKOWITZ. 1988. Forecasting annual geophysical time series. Int. J. Forecasting 4: 103–115.

RALPH, C.J., AND J.M. SCOTT (ED.) 1981. Estimating numbers of terrestrial birds. Stud. Avian Biol. No. 6. Allen Press, Lawrence, Kans.

RICE, J.C. 1978. Ecological relationships of two interspecifically territorial vireos. Ecology 59: 526–538.

RICE, J.C., AND G.T. EVANS. 1988. Tools for embracing uncertainty in the management of the cod fishery in NAFO divisions 2J+3KL. J. Cons. Int. Explor. Mer 45: 73–81.

RICE, J.C., R.D. OHMART, AND B.W. ANDERSON. 1981. Bird community use of riparian habitats: the importance of temporal scale in interpreting discriminant analysis, p. 186–196. *In* D.E. Capen (ed.) The use of multivariate statistics in studies in wildlife habitat. Rocky Mountain Forest and Range Experimental Station. U.S. For. Serv. Gen. Tech. Rep. RM-87. U.S. Department of Agriculture, Fort Collins, Colo.

RICE, J.C., R.D. OHMART, AND B.W. ANDERSON. 1986. Limits in a data-rich model: modeling experience with habitat management on the Colorado River, p. 79–86. *In* J. Verner, M.L. Morrison, and C.J. Ralph (ed.) Wildlife 2000: modeling habitat relationships of terrestrial vertebrates. University of Wisconsin Press, Madison, Wis.

ROTENBERRY, J.T. 1986. Habitat relationships of shrubsteppe birds: even "good" models cannot predict the future, p. 217–222. *In* J. Verner, M.L. Morrison, and C.J. Ralph (ed.) Wildlife 2000: modeling habitat relationships of terrestrial vertebrates. University of Wisconsin Press, Madison, Wis.

SAS INSTITUTE INC. 1982. SAS users guide: statistics, 1982 edition. SAS Institute Inc., Cary N.C. 584 p.

SCHOENER, T.W. 1983. Field experiments on interspecific competition. Am. Nat. 122: 240–285.

SILVERMAN, B.W. 1986. Density estimation for statistics and data analysis. Chapman and Hall, New York, N.Y.

SMITH, S.J., R.I. PERRY, AND L.P. FANNING. 1981. Relationships between water mass characteristics and estimates of fish population abundance from trawl surveys. Environ. Monit. Assess. 17: 227–245.

SOKAL, R.R., AND F.J. ROHLF. 1973. Introduction to biostatistics. W.H. Free man, San Francisco, Calif. 776 p.

TAPIA, R.A., AND J.R. THOMPSON. 1978. Nonparametric probability density estimation. Johns Hopkins University Press, Baltimore, Md.

TERRELL, J.W. (ED.) 1984. Proceedings of a Workshop on Fish Habitat Suitability Index Models. U.S. Fish Wildl. Serv. Biol. Rep. 85(6): 393 p.

TILMAN, D. 1982. Resource competition and community structure. Monogr. Popul. Biol. No. 17. Princeton University Press, Princeton, N.J. 296 p.

VERNER, J., M.L. MORRISON, AND C.J. RALPH (ED.) 1986. Wildlife 2000: modeling habitat relationships of terrestrial vertebrates. University of Wisconsin Press,Madison, Wis. 470 p.

WALTERS, C.J. 1985. Bias in the estimation of functional relationships from time series data. Can. J. Fish. Aquat. Sci. 42: 147–149.

WEGMAN, E.J. 1972. Nonparametric probability density estimation: I. A summary of available methods. Technometrics 14: 533–546.

WIENS, J.A. 1981. Single-sample surveys of avian communities: are the revealed patterns real? Am. Nat. 117: 90–98.

WIENS, J.A. 1985. Habitat selection in variable environments: shrubsteppe birds, p. 227–251. *In* M.L. Cody (ed.) Habitat selection in birds. Academic Press, New York, N.Y.

WILLIAMS, B.K. 1981. Discriminant analysis in wildlife research: theory and applications, p. 50–71. *In* D.E. Capen (ed.) The use of multivariate statistics in studies of wildlife habitat. Rocky Mountain Forest and Range Experimental Station. U.S. For. Serv. Gen. Tech. Rep. RM-87. U.S. Department of Agriculture, Fort Collins, Colo.

WILLIAMS, B.K. 1983. Some observations on the use of discriminant analysis in ecology. Ecology 64: 1283–1291.

WILLIAMS, B.K., AND K. TITUS. 1988. Assessment of sampling stability in ecological applications of discriminant analysis. Ecology 69: 1275–1285.

WORTON, B.J. 1989. Kernel methods for estimating the utilization distribution in home-range studies. Ecology 70: 164–168.

YAKOWITZ, S.J. 1985. Nonparametric density estimation, prediction, and regression for Markov sequences. J. Am. Stat. Soc. 80: 215–221.

YAKOWITZ, S.J., AND F. SZIDAROVSZKY. 1985. A comparison of Kriging with nonparametic regression methods. J. Multivar. Anal. 16: 21–53.

Foraging Flexibility in Pumpkinseed (*Lepomis gibbosus*): Influence of Habitat Structure and Prey Type

J.D. Kieffer[1] and P.W. Colgan[2]

Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada

Kieffer, J.D., and P.W. Colgan. 1993. Foraging flexibility in pumpkinseed (*Lepomis gibbosus*): influence of habitat structure and prey type. Can. J. Fish. Aquat. Sci. 50: 1699–1705.

Foraging behaviour of pumpkinseed (*Lepomis gibbosus*) was investigated in feeding arenas containing different habitat structures and prey types in two sets of experiments, each of three successive 10-d blocks. (A) Two groups of eight foraged in open-water habitats on different prey items: group 1 was presented with prey in the order white worms, fruit flies, white worms and group 2 with fruit flies, white worms, fruit flies. Both capture rate and success increased with successive trials for both prey types, indicating that foraging efficiency increased with experience. Shifts from one prey type to another changed performance; however, overall foraging efficiency did not change dramatically when fish were shifted back to feeding on a previously encountered prey type. (B) Two groups of eight foraged on white worms: group 1 in the habitat order open water, structured, open water and group 2 in structured, open water, structured. Fish fed faster in an open-water habitat but were flexible in a structured habitat. When fish returned to feeding in a familiar habitat type, behavioural patterns were very flexible. The results of these two experiments show that the foraging behaviour of pumpkinseed is relatively sensitive to temporally and spatially separated prey and habitat types.

Le comportement de chasse du craptet-soleil (*Lepomis gibbosus*) a été étudié dans des arènes d'alimentation où la structure de l'habitat et le type de proies différaient. Deux ensembles d'expériences ont ainsi été effectués, chacun s'étant déroulé sur trois décades successives. A) Deux groupes de huit crapets ont pu chasser différentes proies en eau libre : le premier groupe s'est vu offrir successivement des enchytrées, des drosophiles, puis des enchytrées et le deuxième groupe des drosophiles, des enchytrées, puis des drosophiles. Le taux et le succès des captures ont augmenté avec le nombre d'essais successifs à l'égard des deux types de proies, ce qui montre que l'efficacité de la prédation augmente avec l'expérience. La performance a été modifiée avec le passage d'un type de proie à l'autre; toutefois, l'efficacité globale n'a pas changé considérablement lorsque les poissons ont été alimentés de nouveau avec un type de proies connues. B) Deux groupes de huit poissons ont été nourris d'enchytrées : le premier groupe successivement en eau libre, en habitat structuré, puis en eau libre et le deuxième groupe en habitat structuré, en eau libre, puis en habitat structuré. Les poissons se sont nourris plus vite en eau libre, mais se sont vite adaptés à l'habitat structuré. Après leur retour en habitat connu, leur comportement était très souple. Ces deux expériences montrent que le comportement de chasse du crapet-soleil est relativement sensible aux types de proies et d'habitat distincts à l'échelle spatio-temporelle.

Received August 17, 1992
Accepted April 28, 1993
(JB600)

Reçu le 17 août 1992
Accepté le 28 avril 1993

Because many environments fluctuate both spatially and temporally, behavioural patterns of animals should be flexible (see Kamil and Yoerg 1982). Being flexible implies that animals have developed certain mechanisms, such as learning and/or search images, that permit them to alter their behaviour to changing and repeating structural environments and levels of prey types. Features of such flexibility are often influenced by both intrinsic and extrinsic factors and have been widely documented in various species of fish (see Dill 1983 for a review; Ehlinger 1989a).

A number of studies have examined both the long- and short-term modifications in the foraging behaviour of fishes. For example, Keast (1978) showed that there is seasonal (long-term) variability in the types of prey items consumed by fish. Thus, fish may be flexible in their ability to track different prey with time (also see Chapman et al. 1989). Similarly, short-term flexibility has been demonstrated under laboratory conditions (Ehlinger 1989b) in golden shiner (*Notemigonus crysoleucas*), which use two separate foraging modes when feeding on zoo plankton: pump feeding on small zooplankton and particulate feeding on large zooplankton. Many of these studies, however, examine short- or long-term flexibility separately, and only demonstrate flexibility with a change in either habitat structure or prey item. In a previous study (Kieffer and Colgan 1991), we showed that habitat structure influenced the learning ability and foraging rate in pumpkinseed (*Lepomis gibbosus*) and that through learning, pumpkinseed became more efficient foragers. In the present study, we extended our previous results by examining separately the influence of both habitat and prey type on both short- and long-term flexibility in foraging pumpkin seed. Because many foraging environments are complex, and food items change spatially or temporally, the need to learn consecutive tasks when foraging would be common in nature.

Specifically, we addressed two major questions: (1) Are pumpkinseed flexible in their foraging behaviour when pre-

[1]Author to whom correspondence should be addressed.
[2]Present address: Canadian Museum of Nature, Station D, Box 3443, Ottawa, ON K1P 6P4, Canada.

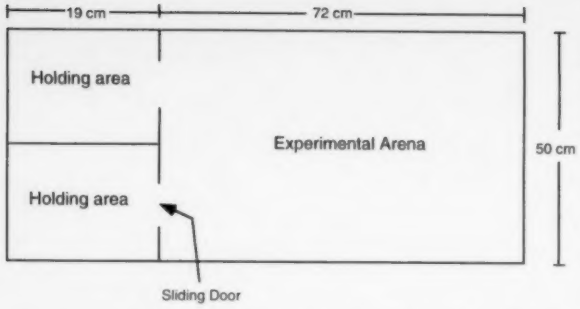


FIG. 1. Schematic view looking down on an experimental aquarium.

sented with different (i) habitat structures or (ii) prey types on different occasions? (2) Do pumpkinseed forage as efficiently (and use a similar pattern of foraging) in a habitat or on a prey type that they have previously experienced but not had access to for a period of 10 d (i.e., "Is an adaptive change in behaviour forgotten after a 10-d period without use?") A period of 10 d was used because this has been shown to be sufficient for a fish to learn a foraging task (Gotceitas and Colgan 1988; Kieffer and Colgan 1991) and some species of fish begin to forget a foraging task in trials separated by several (i.e., ≤8) days (see Croy and Hughes 1991a). Because certain characteristics of habitat structure and prey types often influence the ease with which prey items can be found and captured, we chose to keep either the habitat structure (experiment 1) or the prey type (experiment 2) constant. This allowed us to test separately the effects of a particular prey type or habitat structure on the foraging flexibility of pumpkinseed.

Methods and Materials

Collection and Care of Animals

Pumpkinseed were collected from Lake Opinicon, Canada (see Kieffer and Colgan 1991). While in holding tanks (165 L), the fish were fed a mixture of frozen brine shrimp (*Artemia salina*) and bloodworms or Tetramin every second day. All fish were inexperienced with respect to the experimental aquaria and food.

Experimental Aquaria

Experiments were carried out in two flow-through tanks measuring 91 × 50 × 30 cm (length × width × height). Each tank was divided into two holding areas measuring 19 × 25 × 30 cm and a foraging arena measuring 72 × 50 × 30 cm (Fig. 1). Four 40-W wide-spectrum fluorescent lights were placed above the tank to provide uniform lighting. Each holding area had a sliding door (9.5 × 7 cm) to allow access into the foraging arena. A single fish was held in each chamber and was visually isolated from the other fish. Water depth was 28 cm, temperature was maintained at 23.3 ± 0.1°C (mean ± SE), and a 12 h light: 12 h dark cycle was maintained throughout the series of experiments.

Experimental Prey and Habitat

Two different prey types were used in experiment 1: white worms (*Enchytraeus albidus*) 9–11 mm in length and adult fruit flies (white-eyed; vestigial-like wings) (*Drosophila melanogaster*) 4–5 mm in length. White worms were used because they are benthic; fruit flies generally float on the water surface. Prey items having these qualities allowed us to test the foraging ability of pumpkinseed feeding on spatially distinct prey items. White worms were cultured in moistened soil and fed moistened bread. Fruit flies were cultured in 250-mL jars and fed a mixture of yeast, agar, and sugar, which was placed at the bottom of the jar.

For experiment 2, each arena represented either an open-water or structured habitat (see Kieffer and Colgan 1991). The open-water habitat contained no structure whereas the structured habitat contained "stems" of green polypropylene rope (5.25 mm in diameter, 30 mm long) at a constant density of 800 stems/m.^{2}. The bottom of the entire arena was covered in 1–2 cm of washed sand interspersed with small stones.

Experiment 1: Prey Type

Individual pumpkinseed (total length 75–80 mm) were randomly selected and placed into one of two groups. Fish in group 1 (N = 8) were fed over three successive 10-d blocks in an open-water habitat: white worms (days 1–10), fruit flies (days 11–20), and white worms (days 21–30). Fish in group 2 (N = 8) faced the same habitat conditions but were challenged in the following order: fruit flies (days 1–10), white worms (days 11–20), and fruit flies (days 21–30).

Experiment 2: Habitat Type

Individual pumpkinseed (total length 75–80 mm) were randomly selected and placed into one of two groups. Fish in group 1 (N = 8) were fed white worms for three successive 10-d blocks in open-water (days 1–10), structured (days 21–30), and open-water (days 21–30) habitats. Fish in group two (N = 8) were offered the same prey but in the following habitat order: structured (days 1–10), open water (days 21–30), and structured (days 21–30).

General Procedure

Before the experiments were carried out, the fish were trained to leave the holding arena and enter the foraging arena (see Kieffer and Colgan 1991 for details). The procedure for each experiment consisted of placing 15 prey items (a number determined by pilot studies) at the water surface (white worms or fruit flies depending on the condition). After a short period of time (~1 min), a single fish in the aquarium was released from its holding chamber into the foraging arena for a 5-min foraging bout. The door of the holding chamber remained open for the duration of the foraging bout. Each fish was tested once per day and fed only while in the foraging arena. All trials were run between 09:00 and 12:00 to reduce any circadian effects. Activities were verbally recorded into a tape recorder during each trial by an observer seated in front of the experimental tank. (The presence of an observer did not appear to disturb the fish.) Three activities were measured: (1) Foraging success (number of captures per number of attacks). Foraging success is affected by the number of misses or regurgitations made by a fish. The ability to make feeding attempts, unsuccessful captures, and regurgitations were important parameters that taken together represented important information regarding foraging success. (2) Capture rate (number of captures per time spent foraging). This measure reflects the feeding intensity of the fish. (3) Time to the fifth capture. This is the time (seconds) that it took to capture the first five prey items. This measure also reflects

feeding intensity. Taken together, these activities reflected the foraging dynamics in fish.

To analyze the behavioural activities, a repeated-measures ANOVA was used with factors of day and block. A day effect indicated that behavioural measures changed over days (i.e., learning). Each block of 10 d was analyzed separately to determine whether there were specific changes in the foraging behaviour (i.e., on a particular prey item or habitat type) with time. A block effect represents the influence of habitat or prey type on the learning of the fish. A day × block interaction indicates that learning followed a different course in different blocks (i.e., feeding in different habitats or when feeding on different prey types). Paired *t*-tests were used to analyze the last 4 d of each block. Therefore, each observation in the pair is based on 4 d of data. This comparison was made to determine if behavioural patterns could be maintained with time. Lastly, the data from blocks 1 and 3 were compared to determine whether pumpkinseed need to relearn to feed on a particular prey item or in a habitat that they previously experienced but not had access to for a period of 10 d (i.e., does learning interference occur?).

Before any analysis, the data for all behavioural measures were transformed to better approximate normality; time to fifth capture was log transformed and capture rate was arc sine transformed. The data for foraging success were nonnormal after transformation; therefore, a Friedman ANOVA was used. One fish in experiment 2 died on the 27th day of the experiment: the data for this fish were removed from all analyses.

Significance for all statistical tests was taken to be at the level of 0.05.

Results

Experiment 1: Influence of Prey Type

Group 1: White worms, fruit flies, white worms

Learning was inferred from a change in behaviour with time when the fish were presented with white worms during block 1 (days 1–10): time to fifth capture decreased ($P < 0.0001$; Fig. 2a, block 1) whereas capture rate ($P < 0.0001$; Fig. 2b, block 1) and capture success ($P < 0.0001$; Fig. 2c, block 1) increased. The shift from white worms to fruit flies in block 2 (days 11–20) produced a marked decline in performance (Fig. 2a and 2b, block 2; $P < 0.004$ for the behavioural measures). Quantitatively, when the fish were initially shifted (i.e., day 11) to feeding on fruit flies, they continued to search and make feeding attempts (9 ± 2.4) (mean ± SE) towards any object that resembled white worms, although they did succeed in capturing a few fruit flies before the end of the 5-min test. This suggests that there was some carry-over of behavioural patterns from one block to another. However, during days 12–20 the fish spent most of their time searching the surface for fruit flies and became more efficient at foraging on the surface prey (see Fig. 2c, block 2). Finally, when the fish were shifted back to feeding on white worms in block 3 (days 21–30, Fig. 2), there were no significant changes over days for the time to fifth capture ($P > 0.04$) or capture success ($P > 0.3$), but capture rate increased ($P < 0.03$). However, there was some carry-over of behavioural patterns after fish were transferred from fruit flies back to white worms (i.e., blocks 2 to 3; see Fig. 2b and 2c). This brief carry-over may reflect the flexible nature of pumpkinseed foraging behaviour. The lack of a significant term for days 21–30 suggests that feeding on white worms was relatively simple, as pumpkinseed had previous experience with them. It may also mean that pumpkinseed possess some short-term memory for white worms.

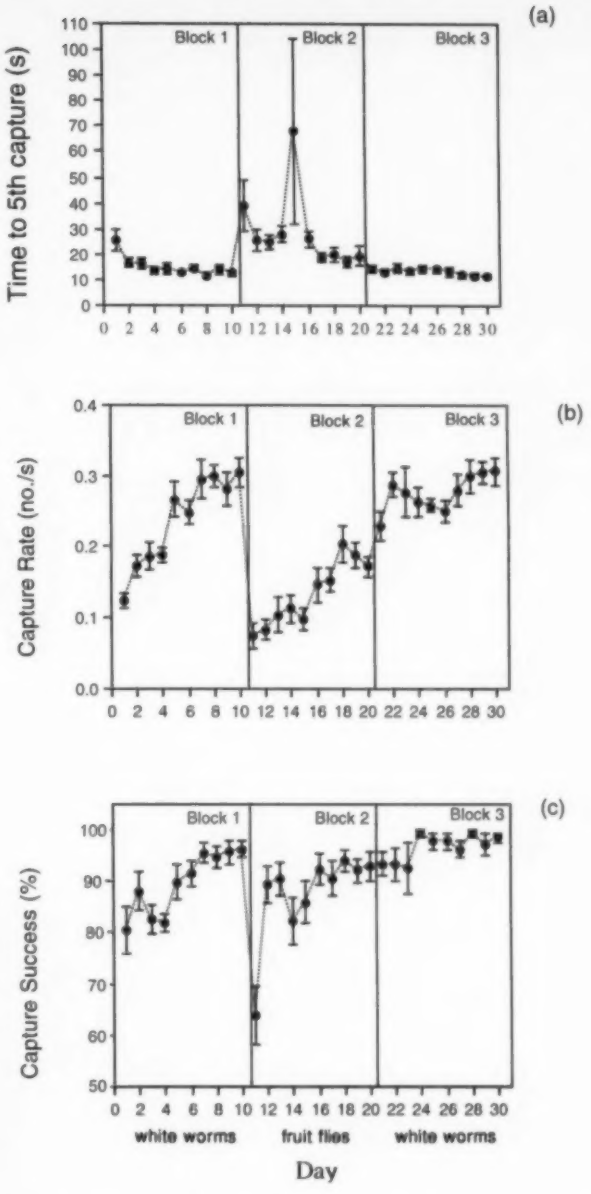


FIG. 2. (a) Time to fifth capture, (b) capture rate, and (c) capture success by pumpkinseed while feeding on white worms (block 1, days 1–10), fruit flies (block 2, days 11–20), and white worms (block 3, days 21–30). Data are means ± SE. The large SE in Fig. 2a (block 2) was the result of an external disturbance on day 15 that was not visible in the other measures.

There was also a significant block effect for the behavioural measures (time to fifth capture, $P < 0.0002$; capture rate, $P < 0.0001$), indicating that behaviour was influenced by the prey type. The block × day term was significant for the behavioural measures, indicating that learning followed

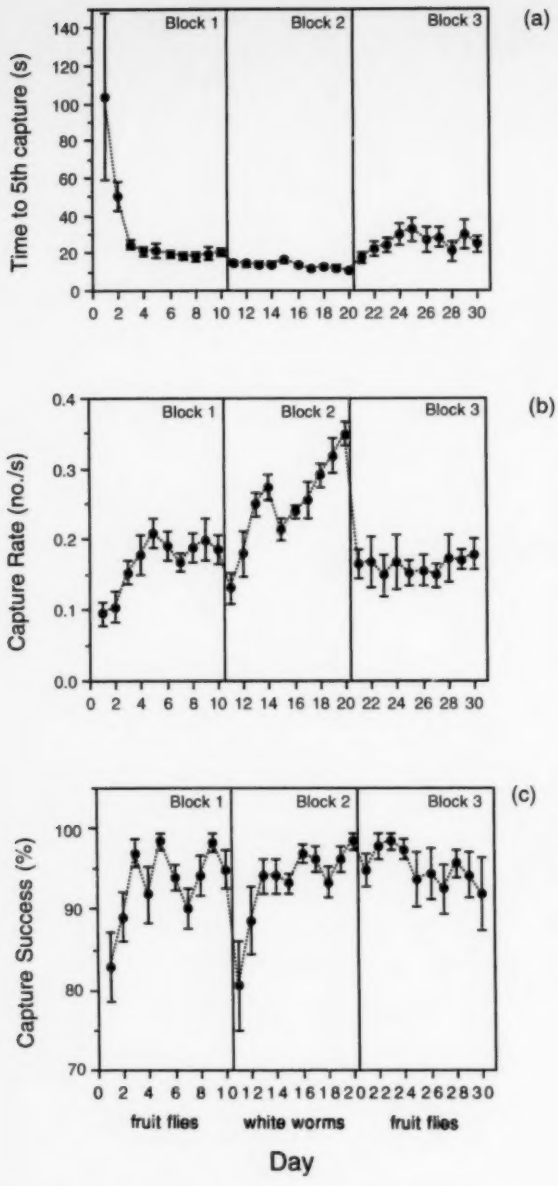


FIG. 3. (a) Time to fifth capture, (b) capture rate, and (c) capture success by pumpkinseed while feeding on fruit flies (block 1, days 1–10), white worms (block 2, days 11–20), and fruit flies (block 3, days 21–30). Data are means ± SE.

different courses when fish were feeding on different prey types. A comparison of the final 4 d of blocks 1 and 3 suggests that the fish were capable of attaining previous levels of foraging for white worms (see Fig. 2). To test whether learning interference occurred, we compared the data from blocks 1 and 3. The presence of a significant block × day interaction for both time to the fifth capture ($P < 0.03$) and capture rate ($P < 0.0001$) demonstrated that the learning curves were different, with improved learning in the third, block (i.e., blocks 1 and 3; Fig. 2a and b), thus reflecting that little or no learning interference was taking place.

Group 2: Fruit flies, white worms, fruit flies

Similar patterns were found when fish were presented with the prey items in the alternative order (e.g., fruit flies followed by white worms and fruit flies). For instance, evidence of learning can be drawn from a change in behaviour with time when the fish were presented with fruit flies during block 1 (days 1–10; $P < 0.0001$, Figs. 3a and 3b; $P < 0.04$, Fig. 3c). The shift from fruit flies to white worms in block 2 (days 11– 20) produced a marked increase in capture rate ($P < 0.006$; Fig. 3b, block 2), a small but significant decrease in time to the fifth capture (Fig. 3a, block 2), and a decrease followed by recovery in capture success (Fig. 3c, block 2). However, when fish were first exposed to white worms (i.e., day 11), they sought out and captured white worms and seldom searched the surface of the water for fruit flies. This may reflect the conspicuous nature of white worms.

After fish were shifted back to feeding on fruit flies for block 3 (days 21–30), capture rate ($P > 0.9$) and capture success ($P > 0.9$) did not change through the following 10 d, but time to fifth capture increased ($P < 0.05$) and became more variable. This latter trend may be the result of the fish looking for the white worms that they experienced in block 2, which may be more profitable prey. Lastly, there appeared to be little carry-over of behavioural patterns between the second and third blocks, unlike what was seen in group 1 fish.

Comparison of the last 4 d of the fruit fly blocks (blocks 1 and 3) established the equivalence of performance with fruit flies as the prey item. Thus, fish were flexible in attaining previous foraging levels (Fig. 3b and 3c), except for the time to fifth capture (Fig. 3a). There was a significant block effect (all blocks) for the behavioural measures ($P < 0.0009$ for time to the fifth capture and capture rate), which suggested that the prey type influenced the foraging behaviour of the pumpkinseed. Also, there was a significant block × day term for the behavioural measures. Lastly, comparison of the data from blocks 1 and 3 implied that the learning curves were different between the two blocks, with the slope being lower in block 3. This suggested that there was some memory for fruit flies, even following a foraging episode with a new prey type (i.e., white worms in block 2).

Comparison of groups 1 and 2

In this experiment, fish had experience with both fruit flies and white worms. However, group 2 fish had prior experience with fruit flies (i.e, block 1). To assess whether previous experience with a particular food item influenced subsequent foraging behaviour, we compared day 21 (start of block 3, Fig. 3) of group 2 with day 11 (start of block 2, Fig. 2) for group 1. Given prior experience with and a second exposure to fruit flies, fish in group 2 (i.e., day 21, block 3) showed lower times to the fifth capture (20 versus 40 s) and both higher capture rates (0.15 versus 0.08) and success (95 versus 63) than fish that did not have previous experience with fruit flies (i.e., group 1 fish). A similar pattern was noted when comparing fish that had previous experience with white worms with fish not having previous experience with white worms (i.e., for all behavioural measures, compare day 21 of Fig. 2 with day 11 of Fig. 3).

Experiment 2: Influence of Habitat Type

This experiment showed trends similar to those of experiment 1. For instance, when the fish were first exposed to white worms in the open-water habitat (days 1–10, block 1),

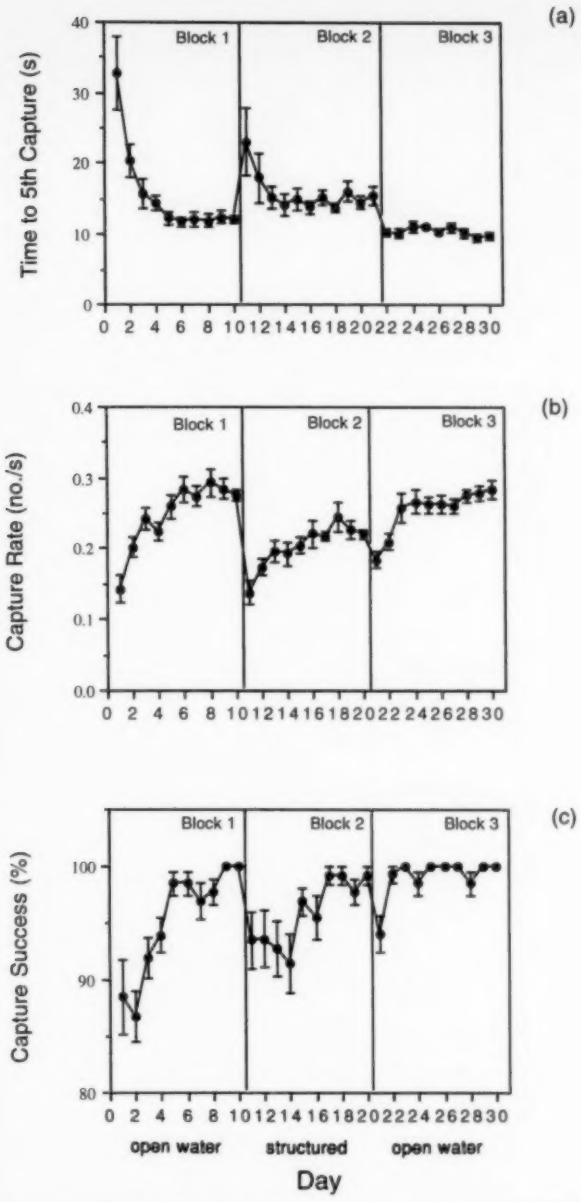


FIG. 4. (a) Time to fifth capture, (b) capture rate, and (c) capture success by pumpkinseed while feeding on white worms in an open-water habitat (block 1, days 1–10), a structured habitat (block 2, days 11–20), and an open-water habitat (block 3, days 21–30). Data are means ± SE.

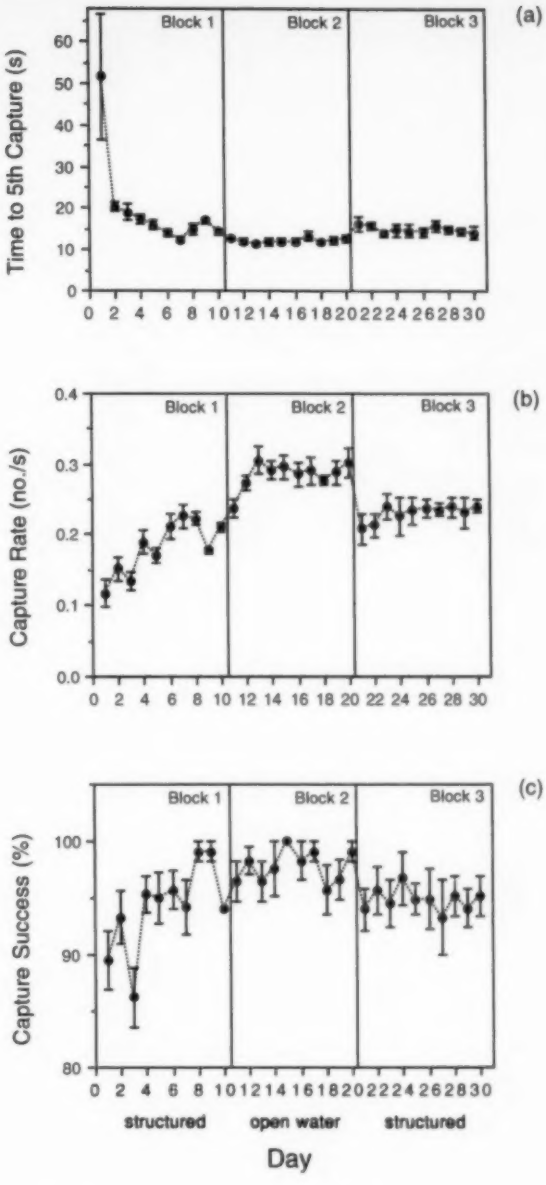


FIG. 5. (a) Time to fifth capture, (b) capture rate, and (c) capture success by pumpkinseed while feeding on white worms in a structured habitat (block 1, days 1–10), an open-water habitat (block 2, days 11– 20), and a structured habitat (block 3, days 21–30). Data are means ± SE.

there was a significant change in the foraging behaviour of pumpkinseed with days (time to fifth capture, capture rate, and capture success; $P < 0.0001$ for all behavioural measures; see Fig. 4, block 1). This was similar for the fish that had their first exposure to feeding on white worms in a structured habitat (see Fig. 5a and 5b, block 1). After transfer to a different habitat (block 2, days 11–20), the behaviour of the fish changed with time; however, they were flexible in adjusting to the new habitat (Fig. 4 and 5, block 2). Overall, the transition from one habitat to another had a significant effect on the behavioural measures. For instance, when fish were transferred from an open-water to a structured habitat (i.e., block 1 to 2, Fig. 4), they had a lower capture rate (Fig. 4b) and took longer to capture five prey items (Fig. 4a). During block 3 (days 21–30), when the fish were returned to the habitat where they had their first experience with white worms (i.e., during days 1–10), the fish reached their final level of foraging quickly. Furthermore, a comparison of the last 4 d of blocks 1 and 3 illustrates the ability of the fish to reach

previously attainable foraging levels (see Fig. 4 and 5). There was a significant block effect (all blocks) and block × day term for both groups of fish. Lastly, comparison of the data from blocks 1 and 3 showed that the learning curves were different between the two blocks (compare blocks 1 and 3, Fig. 4 and 5). Thus, exposure to a new habitat during block 2 did not appear to have a negative influence on the feeding behaviour (i.e., the fish did not have to relearn a feeding task) of pumpkinseed when presented with a prey type in a previously encountered habitat (i.e., during block 3).

Comparison of groups 1 and 2

In this experiment, fish had experience with white worms in both open-water and structured habitats. However, fish in group 2, as opposed to those in group 1, had prior experience with a structured habitat (i.e., block 1, Fig. 5) before encountering white worms in a structured habitat for a second time (i.e., block 3). To assess whether previous experience with a particular habitat influenced subsequent foraging behaviour, we compared day 21 (start of block 3, Fig. 5) of group 2 with day 11 (start of block 2, Fig. 4) of group 1. Given prior experience with a structured habitat (i.e. group 2), fish had lower mean times to fifth capture (18 versus 24 s), higher capture rates (0.20 versus 0.13), and higher success (93 versus 92) than fish that did not have previous experience with a structured habitat (i.e., group 1). However, this was not the situation for fish that had previous experience with white worms in an open-habitat compared with those fish not having a previous encounter with an open-water habitat (i.e., for all behavioural measures, compare day 21 of Fig. 4 with day 11 of Fig. 5). This suggests that previous experience with an open-water habitat does not necessarily confer a greater advantage to foraging sunfish.

Discussion

Day-to-Day Flexibility

Pumpkinseed exhibited day-to-day flexibility (i.e., learning) in their foraging behaviour. For instance, foraging efficiency improved as the number of presentations with the prey types increased (Fig. 2–5, block 1). Therefore, it appears that experience with a particular prey item plays an important role in the increased foraging efficiency. These results are comparable with behavioural measures reported in other species of fish (Ware 1971; Colgan et al. 1991; Croy and Hughes 1991a; Wildhaber and Crowder 1991). As well as facilitating the recognition of novel food items (Kieffer and Colgan 1991), learning may offer other advantages to the fish (see Dill 1983), such as enabling them to ascertain what prey is available in other habitats (see Kamil and Yoerg 1982; Kieffer and Colgan 1992). Learning may also be important because it potentially reduces pursuit and handling time with a specific prey type, thus increasing the net return per unit time. Learning rate may be influenced by the motivational state (i.e., hunger levels) of the animal (e.g., Colgan 1989; Croy and Hughes 1991b).

Task-to-Task Flexibility

Our results also suggest that pumpkinseed are flexible in their ability to change between tasks (e.g., habitat or prey type) on a relatively short time scale. When the fish were transferred from feeding on one prey item to another (i.e., from block 1 to block 2), there was a definite change in performance. For instance, when the fish were transferred from feeding on white worms to feeding on fruit flies, there was an increase in the number of misses (i.e., a decrease in capture success) as well as a decrease in capture rate. It may be that attacking prey items from the water surface requires greater precision with respect to body adjustments, especially since pumpkinseed are more adapted to feeding at the bottom. For example, following the shift from white worms to fruit flies (i.e., day 11), it was noted that pumpkinseed foraged using uncoordinated movements, and some fish only captured a few of the prey items. However, by the fifth presentation (i.e., day 15) with fruit flies, many of the individuals were spending most of their time swimming near and foraging at the surface. Similarly, after a short period of time (i.e., the first few days), pumpkinseed appeared to be fixating on the prey visually.

Foraging flexibility and efficiency are often influenced by the backgrounds in which prey are presented (Clarke and Sutterlin 1985), and hence by how easily prey items are perceived (Jackobsen et al. 1987). For instance, the jerky body movements of white worms on a slighly darkened benthos may enhance the capture rate by pumpkinseed. Alternatively, the translucent bodies of fruit flies on the well-lit water surface may be more cryptic. As a result, when fish were transferred from a white worm to a fruit fly task (i.e., day 11), they have been less flexible than fish experiencing prey items in the reverse order (i.e., fruit flies to white worms; also see Kieffer and Colgan 1991). Also, features of the prey items may reflect the capture tactic. For instance, when feeding on white worms, pumpkinseed used a suction technique to ingest the prey whereas when fish were feeding on fruit flies at the water surface, they used a "gulping-like" feeding technique. This apparent change in the capture "style" between the different prey types by the fish may account for some of the differences in efficiency and flexibility (i.e., learning). Although data on reactive distances and swimming speeds were not collected for fish feeding on either prey item, pumpkinseed appeared to have a much larger reactive distance and swam faster when foraging on white worms. Furthermore, as the fish gained foraging experience, it appeared that they responded to familiar prey from a greater distance. Similar conclusions have been suggested by Ware (1971) and Croy and Hughes (1991a). Furthermore, our results agree with Ehlinger's (1989b) study in which golden shiner switched between filter feeding (i.e., on *Bosmina*) and particulate feeding (i.e., on *Daphnia*) on a very short time scale (for other examples, see Vinyard 1980).

Persistence of Performance: Long-Term Flexibility

When comparing the performance of the fish between blocks 1 and 3, the fish did not return to baseline efficiency during the intervening 10 d. Thus, the values of the behavioural measures began closer to their asymptotic values at the beginning of block 3 (see Fig. 2–5 (except Fig. 2b and 3a)). This is in contrast with the findings of Croy and Hughes (1991a) using fifteen-spined stickleback (*Spinachia spinachia*) and Ware (1971) using rainbow trout (*Salmo gairdneri* = *Oncorhynchus mykiss*). Croy and Hughes (1991a) demonstrated that fifteenspined stickleback retained learned skills on successive days but began to forget them after about 2 d, depending on the type of prey. They also found that handling times for prey offered in trials separated by 8 d were very similar to those of naive fish. Similarly, Ware (1971) found that after 90 d of deprivation with a particular prey item, rainbow trout were back to naive levels with respect to prey recognition.

However, our interpretation that the performance of the fish did not change between blocks 1 and 3 (i.e., the fish did not return to naive levels) is supported by the results of a study by Colgan et al. (1991). They found that bluegill (*Lepomis macrochirus*) retained a learned response for 90 d. The Colgan et al. (1991) study and the present results suggest that the phenomenon of interference (in which competing responses learned on different occasions interfere with the process of recall) was not operating in our system (e.g., see Mackintosh 1974). However, further research is needed in this area.

The ability to retain a foraging response over 10 d, whether on a specific food item or within a particular habitat type (habitat-specific foraging, see Ehlinger 1989a), may be important because it allows animals to remain flexible with the use of a particular foraging mode or tactic. This may be highly advantageous in the patchy environments that these fish occupy (Mittelbach 1981; Werner et al. 1983). Furthermore, maintaining a foraging response over days and being flexible with different prey items and habitat types may be important especially in populations that exist at northern latitudes where prey items fluctuate on a regular basis (see Keast 1978) and the growing seasons are short. Ultimately, the persistence of behavioural traits over time may influence growth and survival of individuals under natural conditions (Colgan et al. 1991).

It should be noted, however, that behavioural flexibility may only be adaptive in habitats that change with respect to prey abundances. Therefore, in environments (i.e., habitats) that remain relatively stable, it may be more adaptive for animals to possess behavioural rigidity (see Ehlinger 1989a). Thus, behavioural flexibility and learning may be more important for generalist animals than for specialists. Similarily, the ability to adapt behaviourally to a changing environment may bestow some selective advantage. However, this does not necessarily suggest that animals living in stable environments are not flexible and do not possess the capacity to learn. To determine whether there is a trade-off between flexibility and learning by fish in stable habitats, one would need to examine the learning ability of those species of fish occurring in those environments. Future studies are needed to test the learning ability of animals living in stable environments (see Stephens and Krebs 1986).

In general, there is some degree of uncertainty as to the features of the environment that enable an animal to retain a learned response. This may be species specific and/or may be influenced by the prey type or habitat structure (e.g., involving the development of a "search image"; Guilford and Dawkins 1987). In our study, it was shown that the prey and habitat type influenced the ability of a fish to retain a learned response and be flexible. To detail the effect of habitat and prey types on the retention of a learned response, we need to perform experiments with specific time schedules and prey types. Similarly, although foraging flexibility and efficiency may be influenced by experience (i.e., learning) with a specific prey or habitat type, other factors such as competition (i.e., interference), social learning, motivation, predator size, and prey size may be important. Investigation into these areas will prove profitable in our understanding of the behavioural flexibility in fishes.

Acknowledgements

The authors thank Dr. V. Walker and Bernie Dunker for providing us with fruit fly cultures. We also thank Dr. Bruce Tufts for the use of his office space while this manuscript was in preparation. Lastly, we thank the staff at the Lake Opinicon Research Facility, especially Frank Phelan, for allowing us the use of their boats to collect fish. This research was supported by a Natural Sciences and Engineering Research Council of Canada operating grant to P. Colgan.

References

CHAPMAN, L.J., W.C. MACKAY, AND C.W. WILKINSON. 1989. Feeding flexibility in northern pike (*Esox lucius*): fish versus invertebrate prey. Can. J. Fish. Aquat. Sci. 46: 666–669.

CLARKE, L.A., AND A.M. SUTTERLIN. 1985. Associative learning, short-term memory, and colour preference during the first feeding by juvenile Atlantic salmon. Can. J. Zool 63: 9–14.

COLGAN, P.W. 1989. Animal motivation. Chapman & Hall, London. 159 p.

COLGAN, P.W., V. GOTCEITAS, AND J. FRAME. 1991. Individual variation, acquisition, and re-acquisition in a foraging task by juvenile bluegill sunfish (*Lepomis macrochirus*). J. Comp. Psychol. 105: 78–84.

CROY, M.I., AND R.M. HUGHES. 1991a. The role of learning and memory in the feeding behaviour of the fifteen-spined stickleback, *Spinachia spinachia* L. Anim. Behav. 41: 149–159.

CROY, M.I., AND R.N. HUGHES. 1991b. The influence of hunger on feeding behaviour and on the acquisition of learned foraging skills by the fifteen-spined stickleback, *Spinachia spinachia* L. Anim. Behav. 41: 161–170.

DILL, L.M. 1983. Adaptive flexibility in the foraging behaviour of fishes. Can. J. Fish. Aquat. Sci. 40: 398–408.

EHLINGER, T.J. 1989a. Learning and individual variation in bluegill foraging: habitat specific techniques. Anim. Behav. 38: 643–658.

EHLINGER, T.J. 1989b. Foraging mode switches in the golden shiner (*Notemigonus crysoleucas*). Can. J. Fish. Aquat. Sci. 46: 1250–1254.

GOTCEITAS, V., AND P.W. COLGAN. 1988. Individual variation in learning by foraging juvenile bluegill sunfish (*Lepomis macrochirus*). J. Comp. Psychol. 102: 294–299.

GUILFORD, T., AND M.S. DAWKINS. 1987. Search images not proven: a reappraisal of recent evidence. Anim. Behav. 35: 1838–1845.

JACKOBSEN, P.J., G.H. JOHNSEN, AND J.C. HOLM. 1987. Increased growth rate in Atlantic salmon parr (*Salmo salar*) by using a two-coloured diet. Can. J. Fish. Aquat. Sci. 44: 1079–1082.

KAMIL, A.C., AND S.I. YOERG. 1982. Learning and foraging behaviour, p. 325–364. *In* P.P. Bateson and P.H. Klopfer [ed.] Perspectives in ethology. Vol. 5. Plenum Press, New York, N.Y.

KEAST, A. 1978. Feeding interrelation between age groups of pumpkinseed sunfish (*Lepomis gibbosus*) and comparisons with the bluegill sunfish (*L. macrochirus*). J. Fish. Res. Board Can. 35: 12–17.

KIEFFER, J.D., AND P.W. COLGAN. 1991. Individual variation in learning by foraging pumpkinseed sunfish, *Lepomis gibbosus*: the influence of habitat. Anim. Behav. 41: 603–611.

KIEFFER, J.D., AND P.W. COLGAN. 1992. The role of learning in fish behaviour. Rev. Fish Biol. Fish. 2: 125–143.

MACKINTOSH, N.J. 1974. The psychology of animal learning. Academic Press, London. 730 p.

MITTELBACH, G.G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. Ecology 62: 1370–1386.

STEPHENS, D.W., AND J.R. KREBS. 1986. Foraging theory. Princeton University Press, Princeton, N.J. 247 p.

VINYARD, G.L. 1980. Differential prey vulnerability and predator selectivity: effects of evasive prey on bluegill (*L. macrochirus*) and pumpkinseed (*L. gibbosus*) predation. Can. J. Fish. Aquat. Sci. 37: 2294–2299.

WARE, D.M. 1971. Predation by rainbow trout (*Salmo gairdneri*): the effect of experience. J. Fish. Res. Board Can. 28: 1847–1852.

WERNER, E.E., G.G. MITTELBACH, D.J. HALL, AND J.F. GILLIAM. 1983. Experimental test of optimal habitat use in fish: the role of relative habitat profitability. Ecology 64: 1525–1539.

WILDHABER, M.L., AND L.B. CROWDER. 1991. Mechanisms of patch choice by bluegills (*Lepomis macrochirus*) foraging in a variable environment. Copeia 1991: 445–460.

Spectral Characteristics of Salmonid Migratory Routes from Southern Vancouver Island (British Columbia)

Iñigo Novales Flamarique and Craig W. Hawryshyn

Department of Biology, University of Victoria, P.O. Box 1700, Victoria, BC V8W 2Y2, Canada

Novales Flamarique, I., and C.W. Hawryshyn. 1993. Spectral characteristics of salmonid migratory routes from southern Vancouver Island (British Columbia). Can. J. Fish. Aquat. Sci. 50: 1706–1716.

Spectral irradiance was measured from 300 to 850 nm under clear and overcast skies in Saanich Inlet and Trevor Channel (Vancouver Island) using an underwater spectroradiometer. The values obtained were corrected for ocular media transmission and pigment absorption of the four visual pigments in small rainbow trout, *Oncorhynchus mykiss* (UV, short, middle, and long wavelength sensitive), to estimate the light available to stimulate each of these photoreceptors. Light levels were compared with particle counts, Chl *a*, and dissolved organic matter (DOM) concentrations at various sites and with similar parameters previously measured for freshwater systems. There was sufficient light to stimulate all the photoreceptors down to 15 m, which was the limiting depth in Saanich Inlet for UV cone simulation and for stimulation of the β absorption band of middle wavelength cones. UV light levels diminished with increasing DOM and Chl *a* concentrations; however, no statistically discernable relationship was found between UV levels and Chl *a* concentrations with distance from the spawning rivers. Under completely overcast conditions, directionality of the sun's rays is maintained under some types of cloud cover. This indicates that navigation using polarized light may occur under variable atmospheric conditions provided enough polarized light is present in the water column.

Au moyen d'un spectroradiomètre immergé, on a mesuré l'éclairement spectral, de 300 à 850 nm, sous ciel dégagé et nuageux, dans l'inlet Saanich et le canal Trevor (île de Vancouver). Les résultats ont été corrigés pour tenir compte de la transmission lumineuse dans le milieu oculaire et de l'absorption des quatre pigments visuels (sensibles à l'UV et aux longueurs d'onde courtes, moyennes et longues) chez la jeune truite arc-en-ciel (*Oncorhynchus mykiss*). Cette correction visait à estimer la lumière qui parvient à stimuler chacun de ces photorécepteurs. L'éclairement a été confronté au comptage de particules, aux concentrations de Chl *a* et de matières organiques en dissolution (MOD) dans diverses stations, ces paramètres ayant déjà été mesurés dans les systèmes dulcicoles. L'éclairement était assez intense pour stimuler tous les photorécepteurs jusqu'à 15 m, profondeur limite, dans l'inlet Saanich, de la stimulation des cônes sensibles aux UV ainsi que de la bande β des cones sensibles aux longueurs d'ondes moyennes. Le rayonnement UV a diminué en proportion inverse de la concentration de MOD et de Chl *a*; toutefois aucune relation statistiquement remarquable n'a été trouvée entre l'intensité des UV, les concentrations de Chl *a* et la distance à laquelle se trouvaient les frayères. Quand le ciel est totalement couvert, les rayons du soleil restent directionnels sous certains types de nuages. Le poisson pourrait donc garder le cap sous la lumière polarisée en conditions atmosphériques variables, mais seulement si la lumière polarisée présente dans la colonne d'eau est en quantité suffisante.

Received June 17, 1992
Accepted February 16, 1993
(JB525)

Reçu le 17 juin 1992
Accepté le 16 février 1993

Although many researchers have examined the visual capabilities of fishes (Tomita et al. 1967, Yager 1969; Bowmaker and Kunz 1987; Hawryshyn and Harosi 1991), few relate their findings to measured photic conditions in the environment (but see McFarland and Munz 1975a, 1975b; Muntz and Wainwright 1978; Heinerman and Ali 1985, 1989; McFarland 1991). Even in studies where an attempt has been made to associate the visual capabilities of the animal with the light cues present in nature, these have sometimes been approximated because of lack of field measurements (Singarajah and Harosi 1992). Spectral data are particularly scarce for coastal oceanic waters where river discharge and upwellings cause high levels of turbidity. The light levels in such environments differ markedly from those found in the open ocean where most measurements have been made (Jerlov 1976; Smith and Baker 1979). Nevertheless, young and adult salmonids must navigate through coastal waters to find their way to and from the open ocean.

Migrating salmon appear to use a number of sensory mechanisms to guide their movements (Quinn 1990), of which olfaction seems to be the primary one for adult salmonids navigating through coastal areas (Quinn and Dittman 1990). However, evidence also exists for magnetic and celestial navigation (Groot 1965; Quinn 1980). Recently, several studies suggest that UV photosensitivity may play an important role in polarized light navigation (Hawryshyn and McFarland 1987; Hawryshyn 1992).

Salmonids possess UV cones (Bowmaker and Kunz 1987) and are sensitive to UV light (Hawryshyn et al. 1989). Two major advantages have been associated with this visual capability: (1) enhanced foraging performance (recently demonstrated for young salmonids in laboratory conditions by Browman et al. 1992) and (2) orientation using underwater UV polarized light (salmonids orient to the E-vector of polarized light in laboratory conditions; Hawryshyn et al. 1990). The first advantage arises

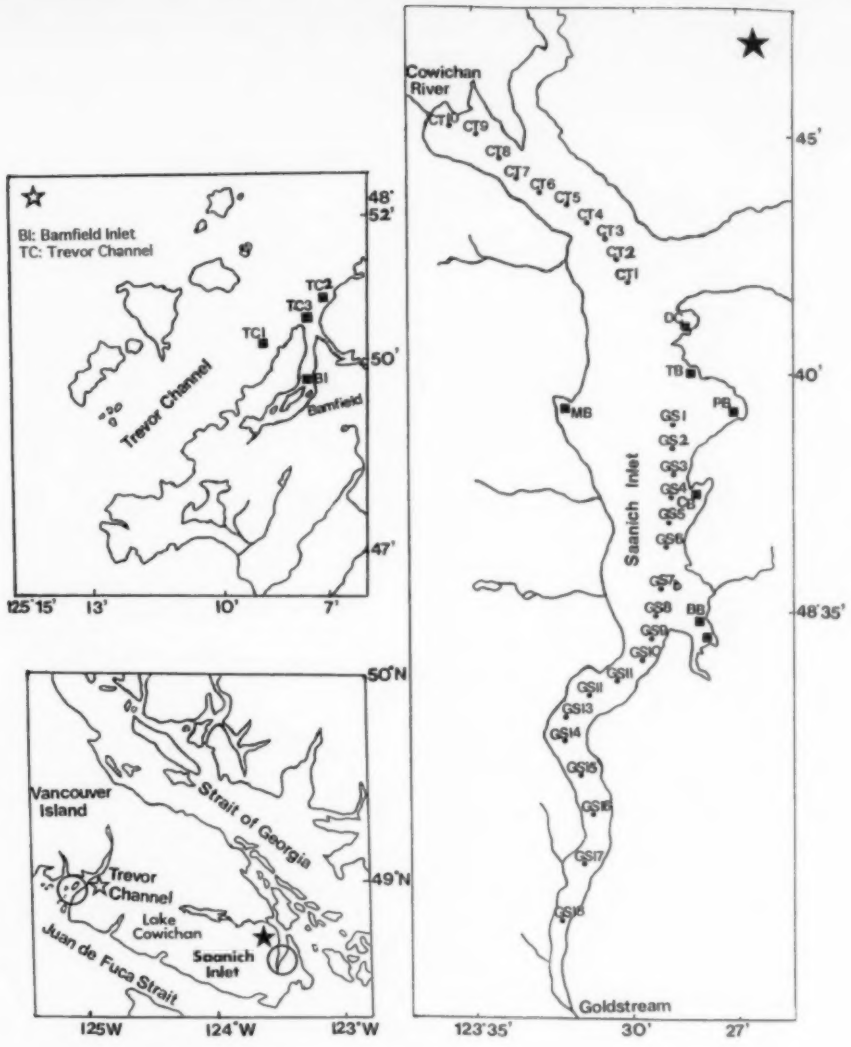


FIG. 1. Study sites in Saanich Inlet and Trevor Channel (position of Lake Cowichan also indicated). GS and CT black dots represent stations along Goldstream and Cowichan Bay transects. Black squares are the stations for which complete sets of spectral irradiance data with depth were obtained.

because water molecules scatter UV light whereas some plankton prey absorb or scatter these wavelengths more strongly (Bowmaker and Kunz 1987), providing a high contrast between plankton target and water background (in the case of higher absorption, the target will appear darker on a UV scattering background, the opposite in the case of higher scattering). The second advantage is a consequence of the scattering of sunlight by water molecules and dissolved particles. Incident light on "Rayleigh-type" particles (i.e., particles with radii smaller than approximately 1/10 of the incident light wavelength) will be scattered in a "donutlike" shape around the particle dipole (Mie theory; Van De Hulst 1957). The light scattered normal to the dipole (i.e., at 90° to the direction of propagation of the incident light) will be theoretically 100% polarized (Rayleigh 1889). Thus, since the most polarized plane of scattered light is approximately 90° with respect to the position of the sun (Waterman 1954), salmonids should always be able to determine the position of the sun provided directionality of the sun's rays is maintained through the atmosphere. Such a sensory mechanism, when combined with a clock, provides salmonids with a "compass" to guide their migrations.

The validity of hypotheses involving the use of UV light for visual tasks is dependent on whether UV light levels in the environment are sufficient to stimulate fish photoreceptors. Our first goal was therefore to measure such light levels in coastal waters of Vancouver Island under different atmospheric conditions (overcast versus sunny) and to compare them with light intensities used in laboratory experiments eliciting fish responses

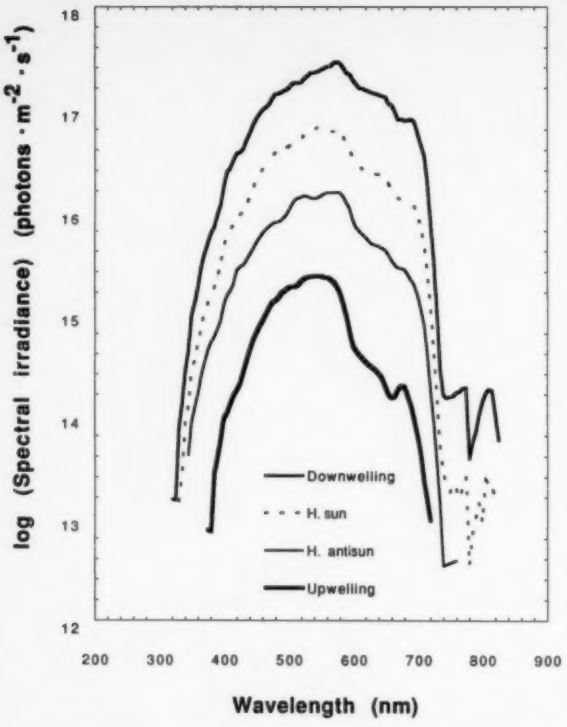


FIG. 2. Spectral irradiance profiles at 3 m depth in Deep Cove for various orientations. Similar profiles were observed at all other depths studied during sunny days. The scans were taken at 13:00 (Pacific Standard Time) on July 16, 1991, under clear skies (sun's elevation: 60°). Upwelling light corresponds to light travelling from the bottom upwards and downwelling from the surface downwards; horizontal (H.) sun is light horizontal to the surface directed away from the sun and H. antisun is light horizontal to the surface directed towards the sun.

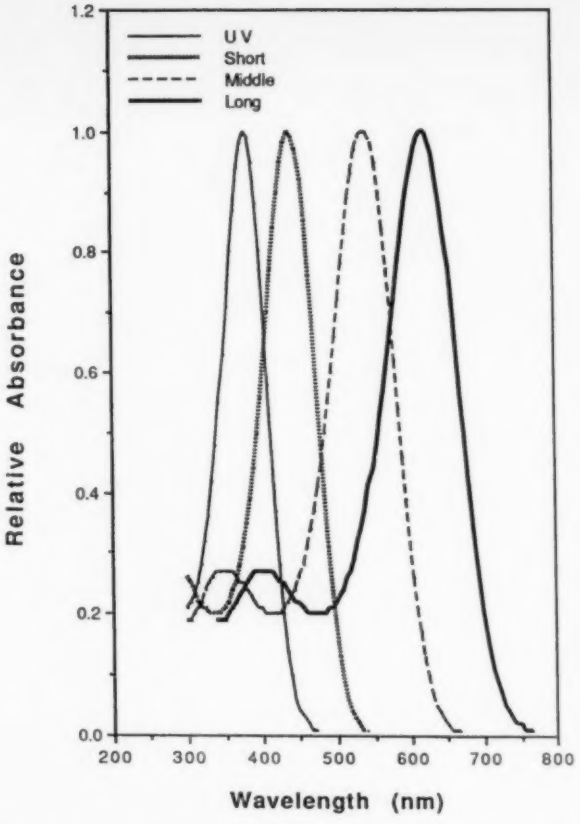


FIG. 3. Absorption curves for the four types of pigments found in the retina of small rainbow trout. These curves were generated from Bernard's (1987 and personal communication) polynomial templates. Notice the secondary absorption bands (β bands) of the middle- and long-wavelength pigments. Their absorption ranges overlap that of the UV cone α band in the region from 300 to 450 nm.

(Hawryshyn et al. 1989). For purposes of migration, we hypothesized that salmonids may be using trends in UV light levels along the coast to guide their movements. To test whether light intensity levels varied with distance from spawning rivers, measurements were taken along transects covering migratory trajectories along the coast. If a trend in UV light levels was found, salmonids could be using it to guide their migration (instead of or along with polarized light detection).

Our second goal was to test the relationship between available light and water chemistry. Dissolved organic matter (DOM) and chlorophyll *a* (Chl *a*) concentrations are the two major biophysical parameters affecting the transmission of UV light through the water column (Prezelin et al. 1991). We therefore correlated light intensities with these two factors. Since similar measurements have been obtained for Lake Cowichan, a salmonid nursery lake (Novales Flamarique et al. 1992), the results in coastal and freshwater environments were compared to detect any photic changes that salmon might experience during their migrations. Such changes can be correlated with ontogenetic alterations in the retinal pigments found in some salmonids (Beatty 1966), which may suggest differences in their visual ecology.

Methods

Spectral irradiance measurements from 18 m to the surface at 3-m intervals were collected at seven locations in Saanich Inlet (east coast of Vancouver Island, B.C., Canada) from July 9 to 26, 1991, and from 23 m to the surface at four locations in Trevor Channel (west coast of Vancouver Island) from August 22 to 31, 1991 (Fig. 1). These locations are on two major migratory routes for Pacific salmonids spawning in Vancouver Island streams.

At each depth in Saanich Inlet, scans were taken in each of four directions: downwelling, upwelling, horizontal sun, and antisun (i.e., light travelling from the surface downwards, from the bottom upwards, parallel to the surface in the direction away from the sun, and parallel to the surface in the direction towards the sun).[1] Each scan collected light from 300 to 850 nm over an angle of 180° (spectral irradiance, Fig. 2). SCUBA divers oriented the spectroradiometer underwater while a researcher on the surface recorded the data through a computer connected to the spectroradiometer. The spectroradiometer was an under-

[1]The sun's position under overcast skies was approximated from past experience during previous dives performed at the same time under clear skies.

water LI-1800 model built by LiCor Inc. (Lincoln, Nebr.), specifications for which are given in Biggs (1984).

To assess how much light was available for stimulation of salmonid photoreceptors at each station, the spectral measurements at each wavelength were multiplied by the corresponding relative absorption values for each of the absorbing photopigments in that region of the spectrum. The absorption values are a measure of the relative ability of each of four types of photoreceptor to catch a photon of a particular wavelength (Fig. 3). The four sets of absorption values (one for each pigment) were deduced from absorbance curves given by Bernard's (1987 and personal communication) polynomial template functions using the equation

$$\text{Absorptance} = 1 - \text{Transmission},$$

where

$$\log_{10}(\text{Transmission}) = -\text{Absorbance}.$$

The spectral irradiance measurements, after correction for photopigment absorption, were multiplied by values giving the percentage of light transmitted through the ocular media of rainbow trout, *Oncorhynchus mykiss* (light through the lens, cornea, and vitreous fluid of the eye; Hawryshyn et al. 1989), since light is absorbed through these layers before reaching the retina. The resulting quantities, integrated over the active absorption range of each pigment, represent the relative proportions of available light for stimulation of each of the cones in the retina of young rainbow trout (<15 g in weight), a quantity that we have termed the "available spectral irradiance" (or "available photon irradiance"; Novales Flamarique et al. 1992).

It should be noted that in the previous calculations the relative absorbances per wavelength as given by Bernard's (1987) polynomial template equation were used to calculate absorptance (Fig. 3). Thus, the available spectral irradiance values are dependent on the absolute absorbance of the pigment at its wavelength of maximum absorption. In order to calculate true absolute absorptance (as opposed to the relative absorbance dependent values presented here and in Novales Flamarique et al. 1992), one must multiply the relative absorbance per wavelength (e.g., from Bernard 1987) by the total absolute absorbance (or optical density) of a photoreceptor along its axis (Harosi 1975). Using published optical density values for amphibians and fish (average value used: 0.0124/μm; Harosi 1975) and multiplying by the average cone outer segment length of rainbow trout (10 μm; F.I. Harosi, personal communication), we arrived at a total absorbance of 0.124 (the outer segment of the cones contains the absorbing pigment). The relative absorbance values generated from Bernard's (1987) polynomial template were multiplied by this value and the true absolute absorptances calculated using the previous formulae. Comparison of these quantities with previous relative absorbance dependent values showed a maximum 9.3-fold difference when the relative absorbance was 0.01; however, values for the most absorbed wavelengths differed from 3 times (for the wavelength of maximum absorbance) to 6.7 times (when the relative absorbance = 0.02) with previous calculations. Available spectral irradiance calculations using these new values for absorptance do not change the threshold depths for photoreceptor excitation or the conclusions reached here. The nonsignificance of the change is due to the following: (1) absorption factors (ranging from 0.002 to 0.24) are multiplied by very large

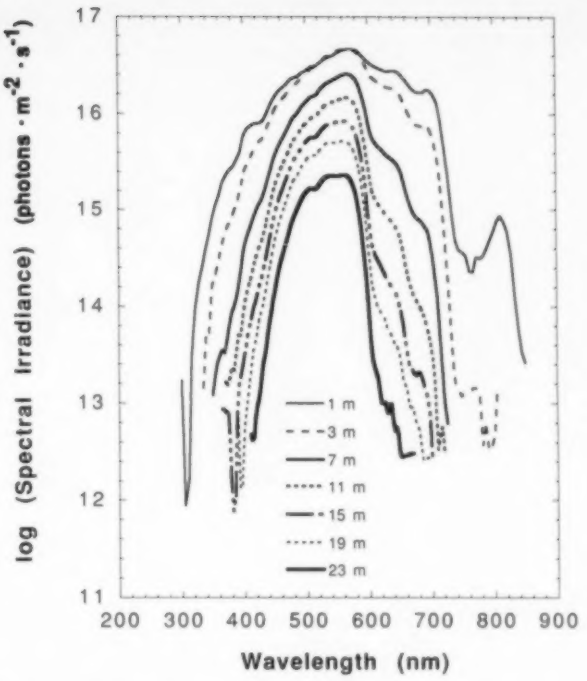


FIG. 4. Spectral irradiance profile for Trevor Channel between 13:05 and 13:30 on August 24, 1991 (TC1 station). Scans were taken under slightly overcast conditions (the sun was visible between and through the clouds; sun's elevation: 30°). Similar profiles were found throughout the duration of the study, with slightly smaller irradiances under fully overcast days (see Fig. 5).

numbers (spectral irradiances $> 10^{13}$ photons $\cdot m^{-2} \cdot s^{-1}$), (2) absorptance results obtained from absolute absorbance values for the highest absorbed wavelengths differ the least with relative absorbance generated results (thus altering little the overall integration results), and (3) the small alterations are within the standard errors for the various types of light at threshold depths.

One of the neuronal mechanisms suggested for detection of UV polarized light requires comparison of the β band retinal outputs from the middle- and/or long-wavelength cone mechanisms and the α band of the UV cone mechanism (Hawryshyn 1992). This is because the α band of the UV cone and the β bands of the middle- and long-wavelength cones have opposite sensitivity to UV polarized light. Comparison of these two different retinal outputs for a given light cue would therefore tell the fish the percent polarization and E-vector orientation of the incident light cue. To test to what depths these secondary bands could be stimulated, further integrations were performed restricting the limits to the β band absorption ranges (300–390 nm, middle-wavelength mechanism; 390–450, long-wavelength mechanism; Fig. 3).

To detect trends in spectral irradiance with distance from freshwater spawning rivers, measurements were taken along paths followed by salmonids spawning at Goldstream Provincial Park and Cowichan River (Fig. 1). Downwelling light measurements at each site were obtained by lowering the spectroradiometer from the side of the boat and holding it at 3 m depth. Water samples were collected and tested for Chl *a* concentrations and particle counts as described below.

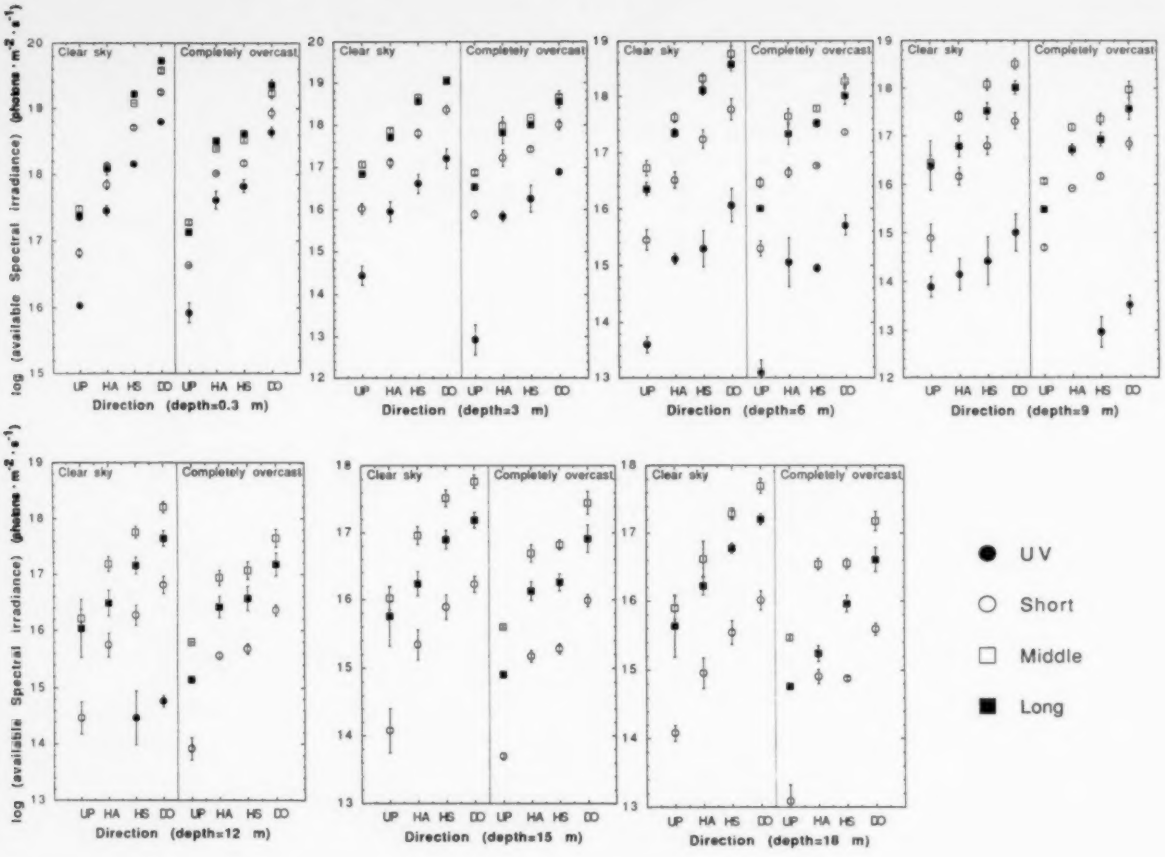


FIG. 5. Log_{10} of the available spectral irradiance with depth and direction for salmonid vision in Saanich Inlet under clear ($n = 3$) and overcast skies ($n = 4$). Bars on symbols are standard errors of the means. Direction symbols denote the following: UP, upwelling light; HA, horizontal antisun; HS, horizontal sun; DO, downwelling light. Note that the scales of available spectral irradiance vary among panels.

To determine whether different weather conditions changed the intensity of light available for salmonid vision, scans at the various locations were taken under clear and cloudy (completely overcast) skies. Since the chemistry of water also plays a major role in the transmission of light through water, Chl *a* analysis of water samples from the various depths was performed on two replicates using the method in Jeffrey and Humphrey (1975). An estimate of the relative amounts of particulate matter at the various locations was also obtained using a Coulter counter (model TA II, Coulter Electronics Inc., Hialeah, Fla.). Using a 70-μm-aperture tube, we counted particles with radii from 0.5 to 12.7 μm in 0.05-mL samples. In addition, DOM estimates were obtained from published Department of Fisheries and Oceans reports (Brown and Borstad 1981). This information permitted us to correlate spectral irradiance with biophysical parameters in the environment.

Because similar measurements have also been made in fresh water systems (Novales Flamarique et al. 1992), comparisons were possible not only among coastal regions but also with lakes. These comparisons may suggest environmental reasons for the varying rhodopsin/porphyropsin ratios in the retina of anadromous migrating fish at different times of the year (Beatty 1966).

Mathematical analysis of results used SAS version 5 edition (SAS Institute Inc. 1987) and LiCor software.

Results

Spectral Measurements

In laboratory experiments, the minimum UV light irradiances required for stimulation of spectrally isolated UV cones using 360- to 380-nm stimulus approximates 10^{13} photons $\cdot$ $m^{-2} \cdot s^{-1}$ (Hawryshyn et al. 1989); this value becomes 6.1×10^{12} photons $\cdot$ $m^{-2} \cdot s^{-1}$ after correction for pigment absorption and transmission through the ocular media. Sensitivity of the fish to other wavelengths may decrease two orders of magnitude in the middle to long wavelengths (Hawryshyn 1991). Correspondingly, the intensities required to stimulate the middle- and long-wavelength cone mechanisms may be up to 100 times those required for the UV or blue cone mechanisms. In the case of UV photoreception, the minimum light levels required, after pigment and ocular media corrections, were found at 20 m depth in Trevor Channel (Fig. 4). In Saanich Inlet, this depth was 15 m (Fig. 5).

The intensity and spectral composition of the available

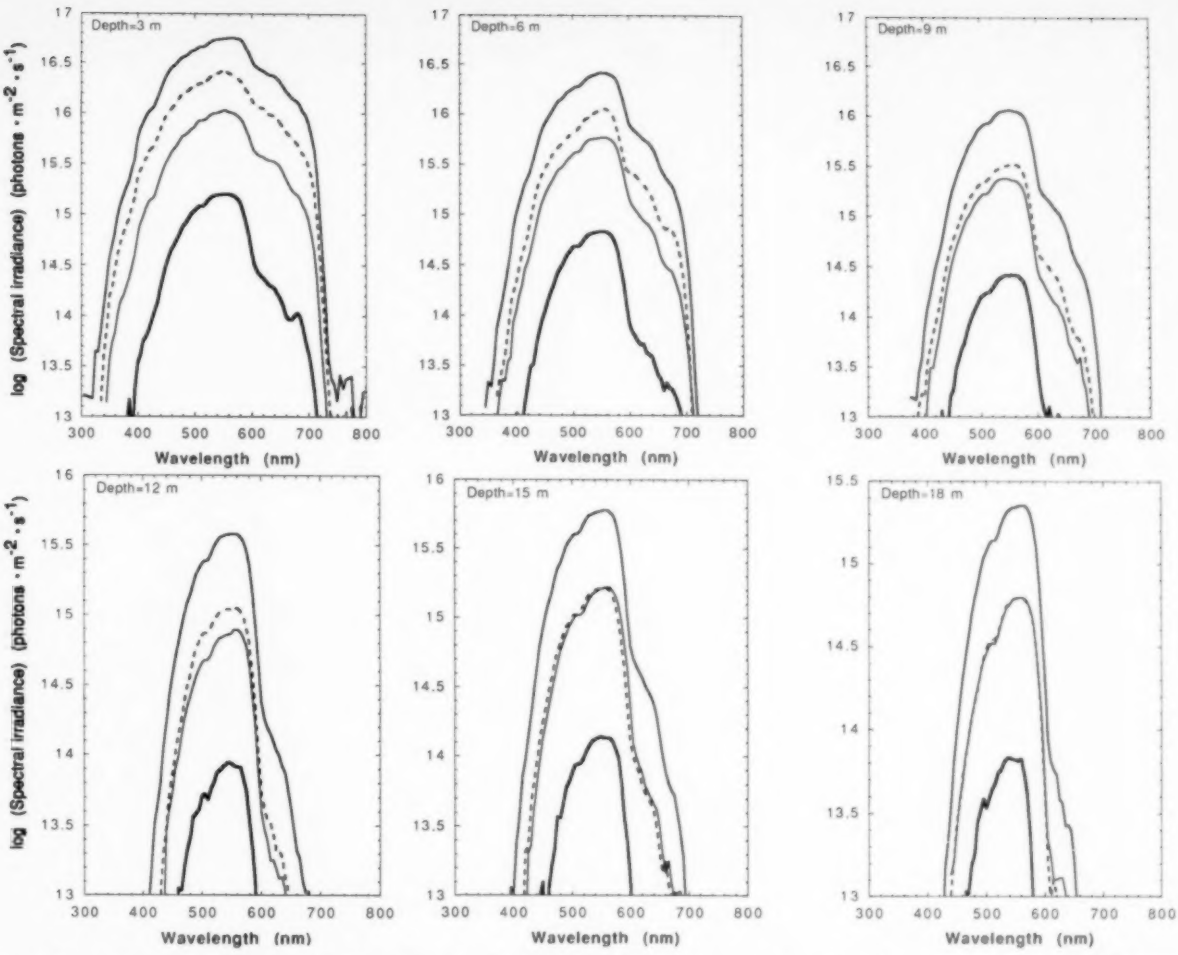


FIG. 6. Spectral irradiance profiles for Mill Bay taken on July 17, 1991, under completely overcast conditions (start of dive: 12:19; end: 12:44 PST). The directionality of the sun's rays was maintained through the cloud layer and detected up to 12 m depth. Decreasing order of light levels as in Fig. 2.

irradiance to stimulate salmonid photoreceptors change with depth and direction of observation (Fig. 5). In Saanich Inlet, long wavelengths dominate the spectrum in surface waters (<6 m depth), but with increasing depth, the "visual" spectrum peaks in middle wavelengths. Light levels decrease in the following manner: downwelling, horizontal sun, horizontal antisun, and up-welling (Fig. 2). This pattern is altered under cloudy conditions because the spectral irradiance differences between horizontal sun and antisun directions tend to vanish. However, such overcast trends depend on the thickness and constituency of the cloud cover (Waterman 1956).

Directionality of the sun's rays under completely overcast skies was observed at up to 12 m depth under some types of cloud cover (Fig. 6). Whether in the presence of clear or cloudy skies, stimulation of the UV photoreceptors in Saanich Inlet is only possible at depths less than 15 m (Fig. 5); this is also the case for stimulation of the β absorption bands of the middle-wavelength photoreceptors (Fig. 7). The relative sensitivities of the β bands are approximately 0.316–0.63 times those of the corresponding α bands (Beauchamp and Lovasik 1973).

The downwelling spectral irradiances for Lake Cowichan, Saanich Inlet, and Trevor Channel show that Lake Cowichan generally has the highest UV levels (Fig. 8). The rapid decrease in UV light levels in Saanich Inlet below 12 m depth coincides with higher Chl *a* concentrations compared with the other study locations (Fig. 9a). Correspondingly, middle wavelengths remain highest at all depths for Saanich Inlet, since they are absorbed minimally by phytoplankton.

Water Chemistry

A comparison between the averaged Chl *a* concentrations in Saanich Inlet and those observed in Trevor Channel showed that both of these coastal waters had more Chl *a* during the summer than Lake Cowichan (Fig. 9a; Brown and Borstad 1981; Novales Flamarique et al. 1992). Furthermore, the first phytoplankton layer was closer to the surface for both coastal waters. Trevor Channel exhibited higher levels of Chl *a* and DOM near the surface than Saanich Inlet (Fig. 9c; Brown and Borstad 1981).

The number of particles between 0.5 and 12.7 μm in radius was higher in Lake Cowichan than in Saanich Inlet (Fig. 9b). Such small-radii-particle differences may indicate the relative

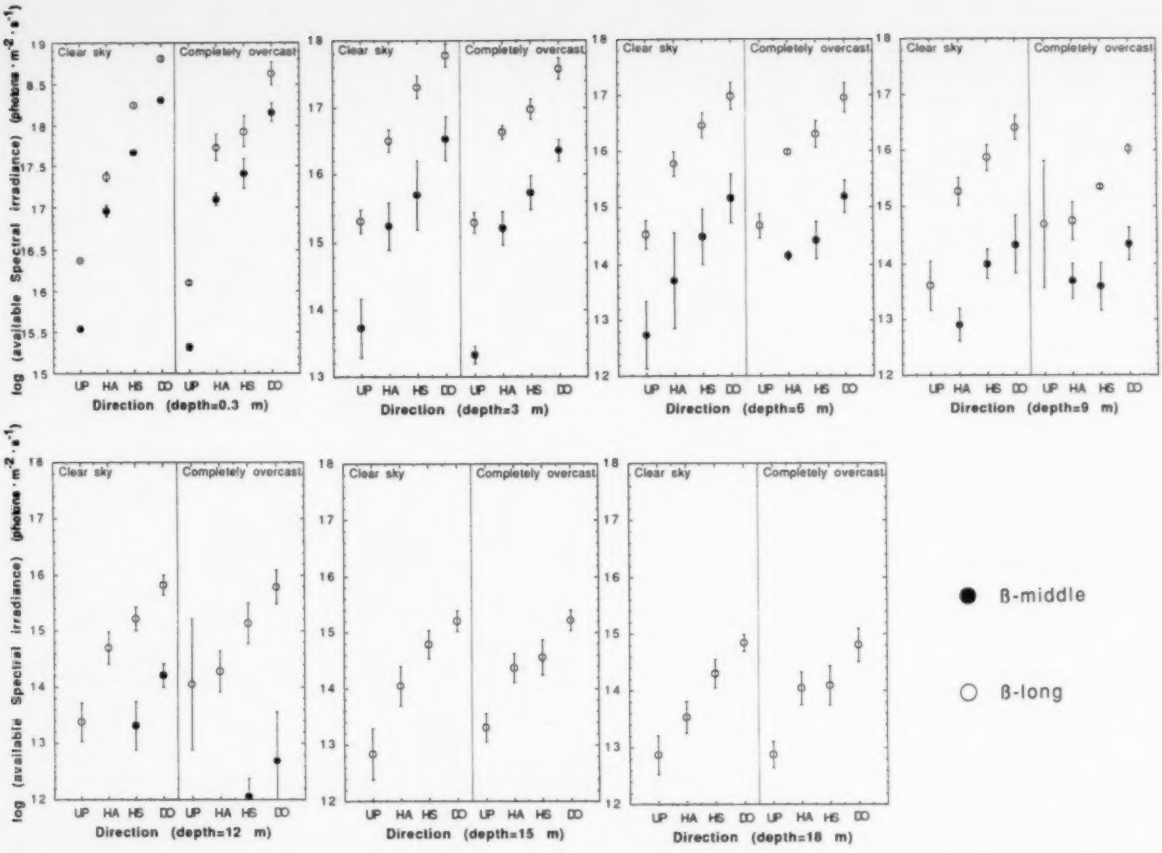


FIG. 7. Log_{10} of the spectral irradiance corrected for ocular media and β band absorption of the middle- and long-wavelength cone mechanisms of small rainbow trout. Direction abbreviations as in Fig. 5. Note that the scales of available spectral irradiance vary among panels.

amounts of polarized light in both environments (Rayleigh 1889). In coastal waters, we observed large numbers of whitish "filament-like" aggregates that would dissociate upon touch and could therefore not be included in the particle counts. However, their surface area, on the order of centimetres, was sufficient to intercept large amounts of radiation.

The number of particles from the various stations in the Goldstream transect generally appeared to decrease with increasing Chl *a* concentrations (Fig. 10). However, there was no significant relationship between intensity of UV light and concentrations of Chl *a* along both transects.

Discussion

Spectral Measurements

Our findings show that salmonids are restricted to surface waters for visual processes involving UV light (<15 m in Saanich Inlet). If target contrast is improved with UV vision, as proposed by Bowmaker and Kunz (1987), this depth restriction would suggest another reason for the periodic movements towards the surface to capture prey. Also, salmonids have been observed to swim near the surface during migrations (Groot 1972; Ruggerone et al. 1990), a time when they may be using UV polarized light for navigation (Hawryshyn 1992).

Navigation using polarized light has been previously associated with perception of scattered light from patches of sky devoid of clouds (Hawryshyn 1992). However, although the sun's rays may not reach the ocean directly under a fully overcast sky, navigation using polarized light may still be possible if the directionality of the incoming solar radiation is maintained through the atmosphere (Waterman 1956). This is because underwater polarization arises mostly from particle scattering in the water column according to the general Mie theory (Van de Hulst 1957), not from sky polarization (Waterman 1954, 1955). The most pronounced band of polarized light, produced by "Rayleigh-type" particles would occur theoretically at 90° to the line traced by the incoming radiation passing through the observer (Rayleigh 1889). Thus, if fish can discern this pattern of polarized light, they could detect the approximate position of the sun behind the cloud layer. Our results for Saanich Inlet show that directionality of sunlight is maintained under some types of clouds (Fig. 6; possibly water droplet clouds; Wellington 1955) and suggest that polarized light navigation as described above should be possible provided sufficient polarized UV light for use by fish (salmonids) is present.

The similarities in the shape of the spectral profiles and available spectral irradiances for both coastal waters and Lake Cowichan (Fig. 8; Novales Flamarique et al. 1992) suggest that

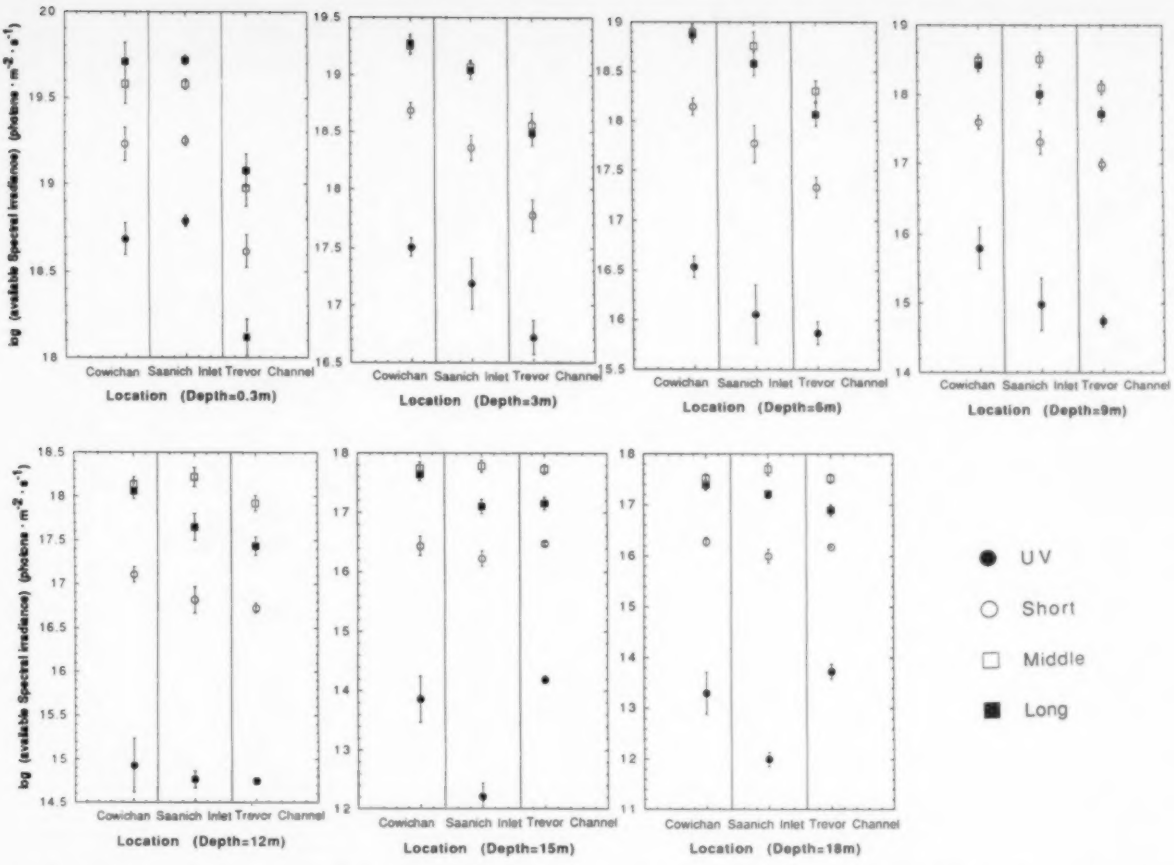


FIG. 8. Comparison of downwelling available spectral irradiance for salmonid vision in Lake Cowichan, Saanich Inlet, and Trevor Channel. Note the higher UV light levels in surface waters of Lake Cowichan. Although more light may penetrate Lake Cowichan, the relative proportions of each type of light remain approximately the same for the three study regions, suggesting similarities in water hue. Particular stations in Saanich Inlet, may, however, appear more red in colour at times during the summer due to red algal blooms. Note that the scales of available irradiance vary among panels.

salmonids should not have to change their retinal pigment ratios to maximize their total photon absorbance from one environment to the other (photoperiods and temperatures being approximately equal in both environments during the fish's migration). The preponderance of middle and long wavelengths in both environments would favour a higher porphyropsin/rhodopsin retinal ratio, since this would increase absorption of longer wavelengths (Wald 1939; Bridges 1972). A change from a rhodopsin-dominated retina (peak absorption at 503 nm) to a porphyropsin-dominated retina (peak absorption at 527 nm) has been observed for returning salmon of some species prior to entering freshwater (Beatty 1966). The pigment adaptation to freshwater has been said to be "anticipated" by the fish; our light measurements show that such an anticipation is possibly the result of the spectral similarities between coastal waters and Lake Cowichan.

Nevertheless, some species such as sockeye salmon, *Oncorhynchus nerka* (and the landlocked sibling species known as kokanee), always have a rhodopsin-dominated retina at all stages in their life (Beatty 1966). This situation may reflect the common occurrence of this species of salmon close to the surface, i.e., in a short wavelength rich environment, throughout the life of its individuals (Pella 1968; French et al. 1976). Rhodopsin-based pigments may help sockeye salmon detect prey more easily in the open ocean by acting as offset pigments (contrast hypothesis, Lythgoe 1968). This situation occurs when the retinal pigment proteins absorb maximally at a wavelength that is not the peak wavelength reflected by the water background and the spectral characteristics of the water background differ with direction of observation (e.g., downwelling versus horizontally scattered light; see McFarland and Munz 1975a). In this case the contrast of a reflective object on the water background (usually composed of longer wavelengths) would be more readily detected than by having a pigment whose maximum absorption matches the background (this because the offset pigment will be less sensitive to the background illumination; McFarland and Munz 1975a). In general, offset pigments reduce photosensitivity to available light but increase contrast of reflective targets (Lythgoe 1968); in contrast, matching pigments increase photosensitivity to available light and are therefore good shadow target detectors. In coastal waters, a rhodopsin-dominated retina would only increase the target detection capabilities of sockeye salmon if the reflecting target, viewed in the horizontal direction, preferentially scatters shorter wavelengths (since downwelling and horizontal irradiance have the same spectral composition;

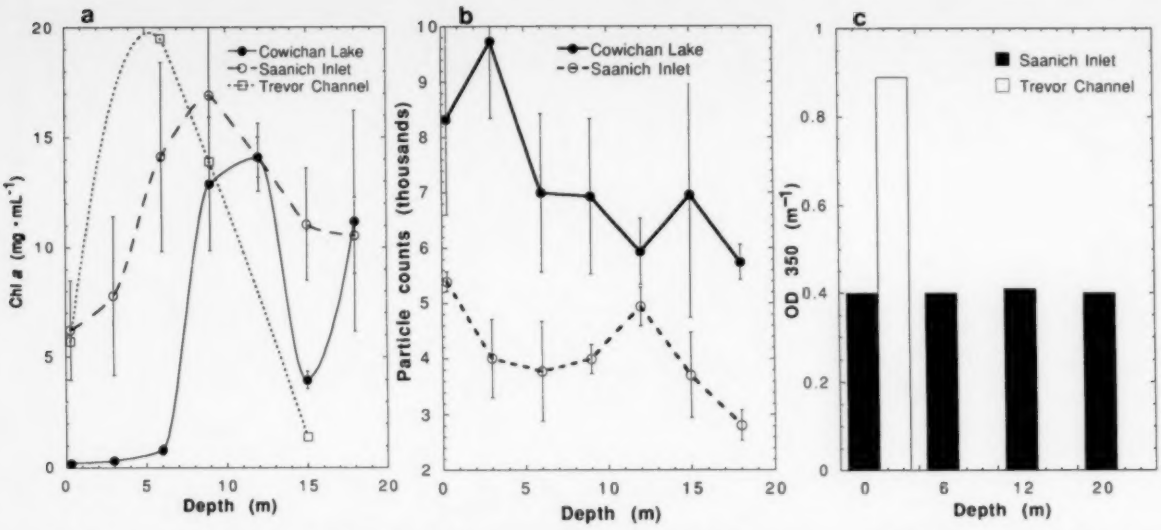


FIG. 9. (a) Chl *a* concentrations with depth in Lake Cowichan, Saanich Inlet, and Trevor Channel. Saanich Inlet and Trevor Channel have higher concentrations of surface Chl *a* than Lake Cowichan. (b) Number of particles between 0.5 and 12.7 μm in radius in Lake Cowichan and Saanich Inlet. (c) Comparison of DOM levels (presented as optical density (OD) data at 350 nm) between Saanich Inlet and Trevor Channel. Chl *a* concentrations for Trevor Channel and all OD measurements were taken from Brown and Borstad (1981). Their study sites V1 and E9 correspond to Saanich Inlet and Trevor Channel, respectively, and their measurements were taken at similar times to ours (July–August).

Fig. 6). This may be the case for schools of small herring that "flicker" as they change orientation in surface waters (these schools being a major source of food for returning salmon; Wing 1977; Healey 1980). The approximate 0.7 log difference between downwelling and horizontal antisun light at 503 and 527 nm (Fig. 6) could also be used by fish with rhodopsin- or porphyropsin-dominated retinae to detect targets on the basis of intensity (the target reflecting downwelling irradiance over a dimmer horizontal background). In this regard, a porphyropsin-dominated retina may act to offset or match the background illumination depending on the spectral characteristics of the water body. However, since the wavelength of maximum absorption of its proteins is higher than that in the rhodopsin-dominated retina, its protein-based pigments would be less offset when operating in coastal waters than those of a rhodopsin-dominated retina (Fig. 6, maximum transmission at 577 nm).

Water Chemistry

The available spectral irradiances found in coastal waters and Lake Cowichan are in good agreement with what would be expected from their biophysical properties. UV light is most abundant in Lake Cowichan as the Chl *a* concentrations are the lowest at this location (Fig. 8 and 9a; Novales Flamarique et al. 1992). Within coastal waters, Trevor Channel has the highest concentrations of DOM and Chl *a* near the surface, which explains the lower UV levels as compared with Saanich Inlet (Fig. 8, 9a, and 9c). However, Saanich Inlet stations averaged higher concentrations of Chl *a* than Trevor Channel for depths greater than 10 m. This may explain the accelerated decrease in UV light for Saanich Inlet at such depths (Fig. 8).

The relative numbers of particles and concentrations of Chl *a* along the Goldstream transect (Fig. 10) may be due to a layer of fresh water resting over the more saline coastal water. Two days of stormy weather preceded these measurements and brownish coloured water from runoff was often detected near the shoreline

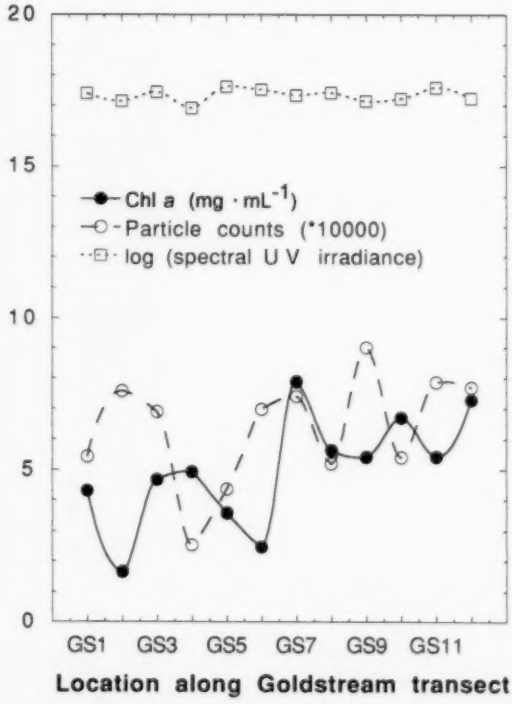


FIG. 10. Chl *a* concentrations, particle counts, and UV levels along the Goldstream transect. Maxima in Chl *a* concentrations appear to match minima in particle counts generally.

(especially as river outflows were approached). Collection of surface samples would thus contain large numbers of particles that may not have mixed yet and reached an equilibrium with the

marine phytoplankton. The small particle sizes counted further indicated these to be runoff nutrients that could be consumed by moderate-sized phytoplankton. This might explain the opposite fluctuations found between particle numbers and Chl *a* concentrations along the Goldstream transect.

UV intensities at the various transect stations tended to decrease with increasing Chl *a* concentrations, but the negative regression between these two variables for the two transects was not significant (F-test probability = 0.6). Similar results have been reported by Gower and Borstard (1990) for Barkley Sound (Vancouver Island). These authors suggested the presence of large and variable amounts of DOM of terrigenous origin to explain the lack of correlation; our results and observations also support this hypothesis. Chl *a* concentrations, like DOM levels along the coast of Vancouver Island, are variable and dependent on many factors such as currents, upwellings, river inputs, and types of catchment area (Pan et al. 1988; Mackas 1992). It is therefore unlikely that once having entered coastal waters, the fish could follow any trends in UV light levels to reach the spawing rivers. This suggests that the fish would be using polarized light patterns (not differences in irradiances) for orientation if using photic cues during migration. Since Chl *a* concentrations during the early spring and late fall are lower on average than those found in this study (Chl *a* concentrations in August are 7 times those in March or April for the Inner Shelf, comparable for Juan de Fuca Strait, and at least 5 times those in October or November for both regions; Pan et al. 1988; Mackas 1992) sufficient light for stimulation of UV photoreceptors should also be available during peak migratory periods. To our knowledge, there is no seasonal information on DOM levels for Vancouver Island waters, but the results of Mackas (1992) for dissolved nitrate show its mean concentrations to be lower during spring and fall than during the summer for Juan de Fuca Strait and lower during the spring but higher during the fall for the Inner Shelf (note, however, the variations during the summer for this region).

In summary, our measurements show that there is sufficient UV light for visually mediated salmonid behaviour to around 15 m depth in coastal waters of Vancouver Island. UV light levels do not follow trends with distance from the spawning rivers but appear to be related to phytoplankton concentrations and DOM levels, themselves variable throughout the coastline. Our results also show that directionality of the incoming light is maintained under certain types of cloud, suggesting that polarized light navigation may still be possible under overcast skies provided sufficient UV polarized light is present in surface waters.

Acknowledgements

Thanks to Andrew Hendry for SCUBA assistance and Dr. Jack Littlepage for exchange of ideas and use of his Coulter counter. Dr. George Mackie kindly provided the boat for the study, and the Institute of Ocean Sciences in Sidney (B.C.) provided moorage. This research was supported by Natural Sciences and Engineering Research Council of Canada and Department of Fisheries and Oceans research grants to C.W.H. I.N.F. was supported by a FCAR scholarship.

References

BEATTY, D.D. 1966. A study of the succession of visual pigments in Pacific salmon (*Oncorhynchus*). Can. J. Zool. 44: 429–455.

BEAUCHAMP, R.D., AND J.V. LOVASIK. 1973. Blue mechanism response of single goldfish optic fibers. J. Neurophysiol. 36: 925–939.

BERNARD, G.D. 1987. Spectral characteristics of butterfly L-receptors using extended Dartnall/McNichol template functions. J. Opt. Soc. Am. Ser. A 2(13): 123.

BIGGS, W.W. 1984. LI-1800UW underwater spectroradiometer instruction manual. Publ. No. 8405–0037. LiCor Inc., Lincoln, Nebr. 126 p.

BOWMAKER, J.K., AND Y.W. KUNZ. 1987. Ultraviolet receptors, tetrachromatic colour vision and retinal mosaics in the brown trout (*Salmo trutta*): age-dependent changes. Vision Res. 27: 2101–2108.

BRIDGES, C.D.B. 1972. The rhodopsin-porphyropsin visual system, p. 417–480. In H.J.A. Dartnall [ed.] Photochemistry of vision. Springer-Verlag, Berlin, Heidelberg, and New York.

BROWMAN, H.I., I. NOVALES FLAMARIQUE, AND C.W. HAWRYSHYN. 1992. Ultraviolet photoreception, prey-search behaviour, and visual acuity in juvenile zooplanktivorous fish. Am. Zool. 45(3): 2. (Abstr.)

BROWN, R.M., AND G.A. BORSTAD. 1981. Canada-France ocean optics experiment: sea-truth data for stations in British Columbia waters. DSS File No. 6-1850-000-000-130, Department of Fisheries and Oceans, Sidney, B.C.

FRENCH, R., H. BILTON, M. OSAKO, AND A. HARTT. 1976. Distribution and origin of sockeye salmon (*Oncorhynchus nerka*) in offshore waters of the north Pacific ocean. Int. North Pac. Fish. Comm. Bull. 34: 113 p.

GOWER, J.F.R., AND G.A. BORSTAD. 1990. Mapping of phytoplankton by solar-stimulated fluorescence using an imaging spectrometer. Int. J. Remote Sens. 11: 313–320.

GROOT, C. 1965. On the orientation of young sockeye salmon (*Oncorhynchus nerka*) during seaward migration out of lakes. Behaviour (Suppl.). 198 p.

GROOT, C. 1972. Migration of yearling sockeye salmon (*Oncorhynchus nerka*) as determined by time-lapse photography of sonar observation. J. Fish. Res. Board Can. 29: 1431–1444.

HAROSI, F.I. 1975. Absorption spectra and linear dichroism of some amphibean photoreceptors. J. Gen. Physiol. 66: 357–382.

HAWRYSHYN, C.W. 1991. Light-adaptation properties of the ultraviolet- sensitive cone mechanism in comparison to the other receptor mechanisms of goldfish. Visual Neurosci. 6: 293–301.

HAWRYSHYN, C.W. 1992. Polarization vision in fish. Am. Sci. 80: 164–175.

HAWRYSHYN, C.W., M.G. ARNOLD, E. BOWERING, AND R.L. COLE. 1990. Spatial orientation of rainbow trout to plane polarized light: the ontogeny of E-vector discrimination and spectral sensitivity characteristics. J. Comp. Physiol. A 166: 565–574.

HAWRYSHYN, C.W., M.G. ARNOLD, D. CHIASSON, AND P.C. MARTIN. 1989. The ontogeny of ultraviolet photosensitivity in rainbow trout (*Salmo gairdneri*). Visual Neurosci. 2: 247–254.

HAWRYSHYN, C.W., AND F. HAROSI. 1991. Ultraviolet photoreception in carp: microspectrophotometry and behaviorally determined action spectra. Vision Res. 31: 567–576.

HAWRYSHYN, C.W., AND W.N. MCFARLAND. 1987. Cone photoreceptor mechanisms and the detection of polarized light in fish. J. Comp. Physiol. A 160: 459–465.

HEALEY, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia, p. 203–229. *In* W.J. McNeil and D.C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, Oreg.

HEINERMANN, P.H., AND M.A. ALI. 1985. Correlation between the photic environment and porphyropsin in the cutlips minnow, *Exoglossum maxillingua*. Naturwissenschaften 72: 488–489.

HEINERMANN, P.H., AND M.A. ALI. 1989. The photic environment and scotopic visual pigments of the creek chub, *Semotilus atromaculatus* and white sucker, Catostomus commersoni. J. Comp. Physiol. A 164: 707–716.

JEFFREY, S.W., AND G.F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*1 and *c*2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz. 167: 191–194.

JERLOV, N.G. 1976. Marine optics, p. 59–61. *In* Elsevier oceanography series. Vol. 14. Elsevier Science Publishing Co., New York, N.Y.

LYTHGOE, J.N. 1968. Visual pigments and visual range underwater. Vision Res. 8: 997–1012.

MACKAS, D.L. 1992. Seasonal cycle of zooplankton off southwestern British Columbia: 1979-89. Can. J. Fish. Aquat. Sci. 49: 903–921.

MCFARLAND, W.N. 1991. Light in the sea: the optical world of elasmobranchs. J. Exp. Zool. (Suppl.) 5: 3–12.

MCFARLAND, W.N., AND F.W. MUNZ. 1975a. The evolution of photopic visual pigments in fishes. Vision Res. 15: 1071–1080.

MCFARLAND, W.N., AND F.W. MUNZ. 1975b. The visible spectrum during twilight and its implications to vision, p. 240–270. *In* G.C. Evans, O. Rockham, and R. Bainbridge [ed.] Light as an ecological factor. Vol. 2. Blackwell Scientific Publications, Oxford.

MUNTZ, W.R.A., AND A.W. WAINWRIGHT. 1978. Annual cycles in the light environments and visual mechanisms of fishes, p. 105–129. *In* J.E. Thorpe [ed.] Rythmic activity of fishes. Academic Press, New York, N.Y.

NOVALES FLAMARIQUE, I., A. HENDRY, AND C.W. HAWRYSHYN. 1992. The photic environment of a salmonid nursery lake. J. Exp. Biol. 169: 121–141.

PAN, D., J.F.R. GOWER, AND G.A. BORSTAD. 1988. Seasonal variation of the surface chlorophyll distribution along the British Columbia coast as shown by CZCS satellite imagery. Limnol. Oceanogr. 33: 227–244.

PELLA, J.J. 1968. Distribution and growth of sockeye salmon fry in Lake Aleknagik, Alaska, during the summer of 1962, p. 45–111. *In* R.L. Burgner [ed.] Further studies of Alaska sockeye salmon. Univ. Wash. Publ. Fish. New Ser. 3.

PREZELIN, B.B., M.M. TILZER, O. SCHOFIELD, AND C. HAESE. 1991. The control of the production process of phytoplankton by the physical structure of the aquatic environment with special reference to its optical properties. Aquat. Sci. 53: 136–186.

QUINN, T.P. 1980. Evidence for celestial and magnetic compass orientation in lake migrating sockeye salmon fry. J. Comp. Physiol. A 137: 243–248.

QUINN, T.P. 1990. Current controversies in the study of salmon homing. Ethol. Ecol. Evol. 2: 49–63.

QUINN, T.P., AND A.H. DITTMAN. 1990. Pacific salmon migrations and homing: mechanisms and adaptive significance. Trends Ecol. Evol. 5: 174–177.

RAYLEIGH, L. 1889. On the transmission of light through an atmosphere containing many small particles in suspension, and on the origin of the blue of the sky. Philos. Mag. 47: 375–384.

RUGGERONE, G.T., T.P. QUINN, I.A. MCGREGOR, AND T.D. WILKINSON. 1990. Horizontal and vertical movements of adult steelhead trout, *Oncorhynchus mykiss*, in the Dean and Fisher channels, British Columbia. Can. J. Fish. Aquat. Sci. 47: 1963–1969.

SAS INSTITUTE INC. 1989. SAS statistics package, version 5 edition. SAS Institute Inc., Box 8000, Cary, N.C.

SINGARAJAH, K.V., AND F.I. HAROSI. 1992. Visual cells and pigments in a demersal fish, the black sea bass (*Centropristis striata*). Biol. Bull. 182: 135–144.

SMITH, R.C., AND K.S. BAKER. 1979. Penetration of U.V.-B and biologically effective dose-rates in natural waters. Photochem. Photobiol. 29: 311–323.

TOMITA, T., A. KANEKO, M. MURAKAMI, AND E.L. PAULTER. 1967. Spectral response curves of single cones in the carp. Vision Res. 7: 519–531.

VAN DE HULST, H.C. 1957. Light scattering by small particles. Dover Publications Inc., New York, N.Y. p. 114–130.

WALD, G. 1939. On the distributions of vitamin A1 and A2. J. Gen. Physiol. 22: 775–794.

WATERMAN, T.H. 1954. Polarization patterns in submarine illumination. Science (Wash., D.C.) 120: 927–932.

WATERMAN, T.H. 1955. Polarization of scattered sunlight in deep water. Deep Sea Res. (Suppl.) 3: 426–434.

WATERMAN, T.H. 1956. Polarized light and plankton navigation, p. 429–450. *In* A.A. Buzzati-Traverso [ed.] Perspect. Mar. Biol. Scripps Institution of Oceanography Symposium.

WELLINGTON, W.G. 1955. Solar heat and plane of polarized light versus the light compass reaction in the orientation of insects on the ground. Ann. Entomol. Soc. Am. 48: 67–76.

WING, B.L. 1977. Salmon food observations, p. 20–27. *In* Southeast Alaska troll log book program: 1976 scientific report. Alaska Sea Grant Rep. 77–11.

YAGER, D. 1969. Behavioral measures of spectral sensitivity in the goldfish following chromatic adaptation. Vision Res. 9: 179–186.

Elevated Levels of Arginine Vasotocin in the Brain of Brook Trout (*Salvelinus fontinalis*) from Acid Lakes: A Field Test of a Potential Biomarker for Acid Stress

A. Hontela

TOXEN, Département des Sciences Biologiques, Université du Québec à Montréal, C.P. 8888, Succursale A, Montréal, QC H3C 3P8, Canada

J.B. Rasmussen

Limnology Research Centre, Department of Biology, McGill University, 1205 Ave. Docteur Penfield, Montréal, QC H3A 1B1, Canada

K. Lederis

Department of Pharmacology, Faculty of Medicine, University of Calgary, Calgary, AB T2N 1N4, Canada

and H.V. Tra and G. Chevalier

TOXEN, Département des Sciences Biologiques, Université du Québec à Montréal, C.P. 8888, Succursale A, Montréal, QC H3C 3P8, Canada

Hontela, A., J.B. Rasmussen, K. Lederis, H.V. Tra, and G. Chevalier. 1993. Elevated levels of arginine vasotocin in the brain of brook trout (*Salvelinus fontinalis*) from acid lakes: a field test of a potential biomarker for acid stress. Can. J. Fish. Aquat. Sci. 50: 1717-1727.

The levels of arginine vasotocin (AVT), an osmoregulatory peptide, were determined by radioimmunoassay in brain tissue of brook trout (*Salvelinus fontinalis*) of a wide size range (50–380 mm) from softwater Laurentian lakes ranging in pH from 5.0 to 6.9 at different seasons. Multivariate models (ANCOVA) were developed to quantify the relationship between AVT, pH, body size, and season. Brain AVT levels increased with body size, and the allometric slope was highest in the low-pH lakes (pH 5.0-5.5). Although brook trout >150 mm had higher brain AVT levels at low pH, no significant differences were detected for brook trout <150 mm. We hypothesize that the sensitivity of brook trout at the parr stage to acid stress may be linked to their inability to mobilize a hormonal response involving AVT. The seasonal variation in brain AVT levels was similar in all the lakes studied, summer levels being the highest. Although this field study revealed that AVT levels depend also on factors other than acid stress (body size and season), our ANCOVA models allow adjustment for the effects of these covariables. Analyses of this type can be used to field test and calibrate biomarkers for use in ecotoxicology.

Au cours de différentes saisons, nous avons dosé par voie radio-immunologique l'arginine-vasotocine (AVT), peptide de la régulation osmotique, dans le tissu cérébral de l'omble de fontaine d'une large gamme de tailles (de 50 à 380 mm), provenant de lacs d'eau douce des Laurentides dont le pH variait de 5,0 à 6,9. Nous avons construit des modèles multivariés (ANCOVA) pour quantifier le rapport entre la concentration de AVT, le pH, la taille du poisson et la saison. La concentration de AVT cérébrale a augmenté avec la taille du poisson, et la pente allométrique était plus forte dans les lacs où le pH était faible (de 5,0 à 5,5). Même si, chez les truites de plus de 150 mm, la concentration de AVT cérébrale était supérieure quand le pH était faible, aucun écart significatif n'a été décelé chez les sujets de moins de 150 mm de longueur. Nous posons que la sensibilité des truitelles au traumatisme de l'acidification peut être reliée à l'absence de réaction hormonale à la AVT. Les variations saisonnières des concentrations de AVT cérébrale étaient semblables dans tous les lacs étudiés, les concentrations étant maximales l'été. Même si l'étude a révélé que les concentrations de AVT dépendent également d'autres facteurs que le traumatisme de l'acidification (taille et saison), nos modèles d'analyse de la covariance permettent de corriger les effets de ces variables. De telles analyses peuvent servir à valider et à étalonner, en conditions réelles, les marqueurs biologiques utilisés en écotoxicologie.

Received July 10, 1992
Accepted March 5, 1993
(JB549)

Reçu le 10 juillet 1992
Accepté le 5 mars 1993

Impaired osmoregulatory function has been identified as a major adverse effect of exposure to acid water in fishes. Fish have been shown to exhibit a range of physiological disturbances including increased permeability of the gills and loss of plasma electrolytes (McDonald and Milligan 1988; Wood et al. 1988a, 1988b), reduced capacity for gas exchange across the gills (Malte 1986), and histopathological changes in the secretory profile of osmoregulatory hormones that mediate ionic and hydric homeostasis. Exposure to acid water increases the activity of prolactin cells (Wendelaar Bonga et al. 1988) and elevates cortisol and decreases thyroid hormone levels (Brown et al. 1984, 1990) in the blood. Chevalier et al. (1986) detected reduced urotensin II levels, osmoregulatory hormones produced in the urophysis, in brook trout (*Salvelinus fontinalis*) from acidified lakes. Subsequently, urotensin I levels were correlated with pH, while urotensin II levels were correlated with total aluminum in the water (Hontela et al. 1989).

The early detection and quantification of acid stress in wild

fish remains difficult, despite great advances, mostly through laboratory studies, in understanding the mechanisms of action as well as the modulating influences of calcium and aluminum ions (Audet et al. 1988; Wood et al. 1988a, 1988b). Changes in life-cycle parameters such as diminished reproductive success (Frenette and Dodson 1984; Lappalainen et al. 1988), diminished growth (Lacroix et al. 1985; Gunn and Noakes 1987), or increased mortality (Watt et al. 1983; Leuven and Pyen 1987) in fish populations from acidified waters have been reported. Yet, physiological parameters that could be used as early warning indicators of impending health problems (biomarkers) caused by exposure of feral fish to acid water have not been clearly identified. Perturbation of plasma electrolytes (Lyons 1982; Lacroix and Townsend 1987) and occurrence of gill pathology (Chevalier et al. 1985; Karlsson-Norrgren et al. 1986) have been documented.

Recently, we reported that the brain levels of arginine vasotocin (AVT), an osmoregulatory peptide from the neurohypophysis, increased in brook trout exposed to sublethal pH (5.6) and total aluminum (300 μg/L) for 1 wk in the laboratory (Hontela et al. 1991). The osmoregulatory functions of AVT include elimination of water by the kidneys and reduction of gill permeability by vasoconstriction of the gill capillaries (Maetz et al. 1964; Lahlou and Giordan 1970; Sawyer 1977; Bennett and Rankin 1986). These parameters are potentially important during the physiological response of fish to low pH.

The purpose of this study is to establish the patterns of spatial and temporal variation in brain AVT levels in brook trout from acidic softwater lakes and to determine whether our laboratory findings (elevated brain AVT in response to acid exposure) can be corroborated in the field. Brain AVT concentrations have never been studied in feral fish, and thus the influences of environmental covariates (e.g., seasonal patterns) that can be controlled in laboratory studies, are unknown. However, before a physiological parameter can serve as an ecotoxicological "biomarker," it is necessary to understand quantitatively the influence of such variables as body size, season, and other environmental parameters on the biomarker (Huggett et al. 1992). One way to examine such effects is to compare brain AVT levels in brook trout over a wide size range in a series of lakes ranging from acid to neutral pH (high to low aluminum levels) across a range of seasons. Such comparative studies can be examined by analysis of covariance (ANCOVA), leading to multivariate models that incorporate the response to the pollutant (acid) as well as to other significant covariates. This approach has recently been used successfully in modelling the bioaccumulation of organic contaminants in lake fishes (Rasmussen et al. 1990; Rowan and Rasmussen 1992). To our knowledge, this is the first study that has attempted to use this type of statistical–comparative approach to "field calibrate" a physiological biomarker.

Methods

Capture and Sampling of the Fish

Brook trout of both sexes (15–20 per lake) were caught in gill nets of two different mesh sizes. A mesh of 2.5 cm (4 × 30 m) was used to capture fish >150 mm long, while a mesh of 6–8 mm (1 × 5 m) was used for fish <150 mm long. Fish were captured from May to September. The nets were set from a canoe (motor boats were not permitted in the study area) and lifted hourly to minimize the time the fish spent in the nets. Fish were stunned immediately after removal from the nets and placed on chipped ice. A blood sample was taken from the caudal vein within 30 min, and the body length and weight of each fish were recorded. The fish were then sacrificed by a spinal section, a U-shaped flap of the cranium was lifted, and the brain was dissected out by cutting the cranial nerves and the optic chiasma. The pituitary, easily visible once the brain was removed, was also dissected out. Both tissues were placed in vials filled with acetone. All dissection was completed within 2 h of capture.

Fish were sampled from mid-May to mid-September, and we classified our sampling times as spring (May to mid-June), the period when the brook trout were active near the surface and in shallow water, and gonads were in the immature state, summer (mid June to late August), the period when the fish retreat to the thermocline and are not active at the surface (gonads recrudescing), and fall (late August to September), the period when surface activity is renewed and prespawning fish begin to congregate in the vicinity of spawning areas. We did not sample fish at the height of spawning, which takes place during October in these lakes.

Study Lakes

Brook trout were sampled in seven lakes in the Réserve Faunique Mastigouche (Fig. 1) in northeastern Québec (46°26°N, 73°09′W). To sample sublethally acid-stressed populations, we selected lakes ranging from near neutral (pH 6.9) to pH 5.0 in an area that receives precipitation with a mean annual pH of 4.4 and exhibits a wide range of natural buffering capacity (Boulet and Jacques 1987; Dupont 1989). The lakes are free of human activity except for hunting and angling. In the first year of the study (September 1988–89), we sampled only the four lakes in the pH range 5.5–6.9 (Pyrolle, Franciscain, Demarest, and de la Loutre); however, in 1990, we added three lakes (Welly, Brochard, and Petit Penang), which we sampled once each. This extended our lower pH limit from 5.5 down to 5.0 and added a strongly coloured lake containing humic matter to the study.

The physicochemical characteristics of the lakes are given in Table 1. Surface water samples were collected in two clean 1-L polyethylene bottles. The pH and the conductivity were immediately measured in the first sample with a field pH meter (Hach). Colour was determined later in the laboratory by spectrophotometry (Rasmussen et al. 1989). The second water sample was acidified to pH 2 with ultrapure HNO_3, and total aluminum and Ca^{2+} were measured by atomic absorption in the laboratory at 309 nm. Surface water temperature was recorded with a thermistor. We estimated monomeric aluminum from total aluminum, pH, and colour using a multiple regression (Table 1) based on US EPA (1986) data from 126 lakes. Monomeric aluminum levels were low (<80 μg/L) even in our most acidic lakes, well below 300–500 μg/L, water concentrations considered toxic to brook trout (Wood et al. 1988a, 1988b).

Laboratory Analysis

The brain (separated into telencephalon, hypothalamus, and hindbrain) and the pituitary gland preserved in acetone were dried under vacuum and then homogenized in 0.25% acetic acid and extracted for 5 min at 100°C. The AVT levels were determined in the pituitary and brain extracts with a radioimmunoassay specific for AVT, as previously described (Hontela et al. 1991).

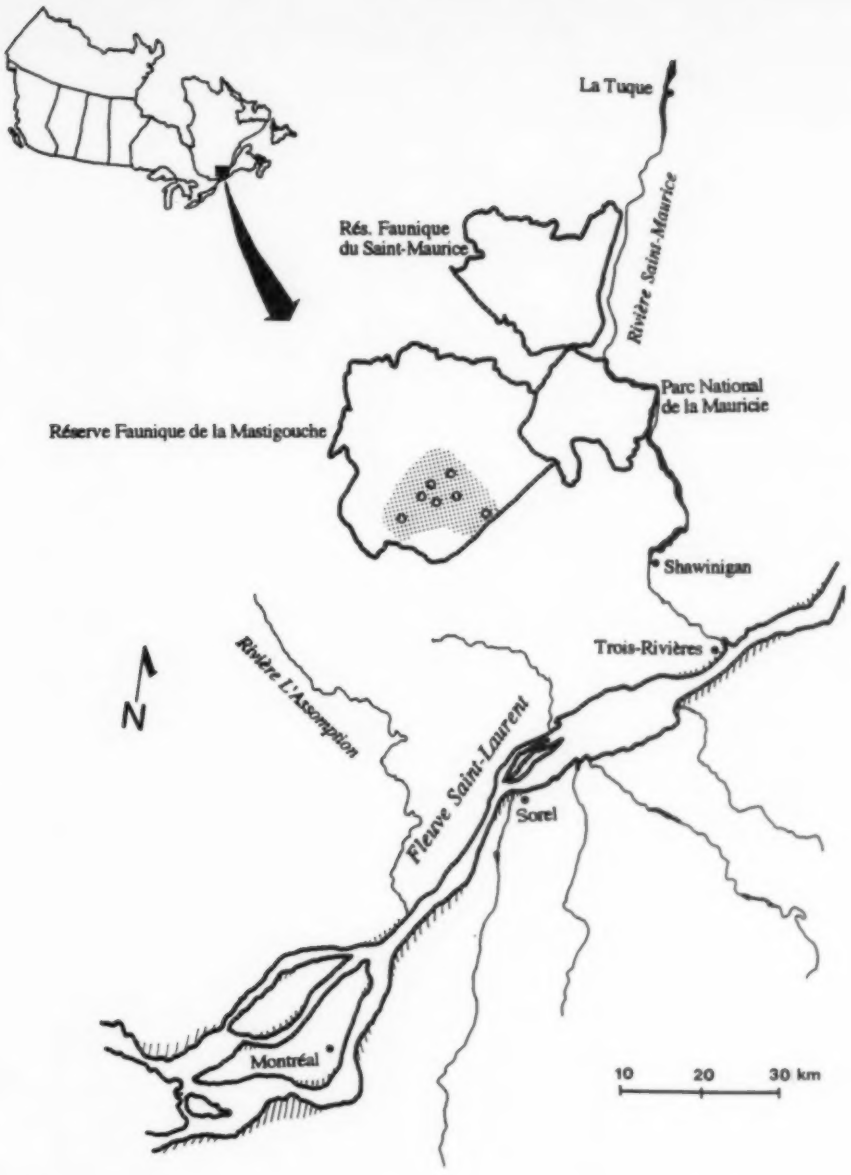


FIG. 1. Map of northeastern Québec and the location of the Réserve Faunique Mastigouche. Brook trout were sampled in acidic and neutral lakes (open circles) situated in the southern portion of the reserve (shaded area).

Statistical Analysis

In an exploratory canonical correlation analysis (MGLH SYSTAT, Wilkinson 1989), we regressed AVT concentrations from four tissues (telencephalon, hypothalamus, whole brain, and pituitary) against a set of regressors including fish length and season (spring, summer, and fall). This exploratory analysis showed that brain AVT (picograms of AVT per milligram of brain tissue) was best related to the predictors and that the AVT levels in the individual brain components and the pituitary all exhibited similar patterns. For this reason, all of the regression analyses outlined in this paper use total brain AVT (picograms of AVT per milligram of brain tissue). Variability among fish in brain AVT levels increased with fish size and thus, it was necessary to log-transform the brain AVT values to achieve homoscedastic residuals. Because log brain AVT was strongly related to body length, we analyzed lake and season (both categorical variates) and interactions as well in the ANCOVA model of MGLH using length as the continuous covariate.

TABLE 1. Characteristics of the lakes sampled.

Lake	Season	pH	Ca^{2+} (mg/L)	Total Al (μg/L)	Monomeric Al (μg/L)[a]	Colour (mg Pt/L)	Conductivity (μS/cm)	Parr per net hour	T(°C)
Brochard	August	5.0	1.3	235	75	16	19	0	11
Welly	June	5.3	1.4	210	65	10	13	0.04	11.5
Pyrolle	May	5.0	2.1	235	38	38	15	—	10
	July	5.3	—	143	—	—	21	—	23
	September	5.5	—	138	—	—	19	0[b]	17.5
Franciscain	May	5.4	1.2	205	29	50	15	—	9.5
	July	5.5	—	205	—	—	18	—	24
	September	5.8	1.8	163	—	—	17	2.2	16
Petit Penang	September	6.2	—	294	21	150	18	2.0	27
Demarest	May	6.0	2.0	72.5	8.5	29	23	—	9.5
	July	6.3	—	64.0	—	—	22	—	23
	September	6.4	2.5	42.5	—	—	20	0.2	16.5
de la Loutre	May	6.6	2.1	69.0	7.5	17	20	—	10
	July	6.9	2.0	57.4	—	—	19	—	22
	September	6.9	2.2	32.5	—	—	21	2.0	17

[a]Log monomeric aluminum = 2.04 + 0.73 log total aluminum − 0.30 pH − 0.28 log colour, $SE_{est} = 0.23$, $R^2 = 0.85$, based on 126 lakes from New York State (US EPA 1986). US EPA method for monomeric aluminum involved 8-hydroxyquinoline addition to unacidified filtered samples followed by extraction with methyl isobutyl ketone.
[b]Presence of minnows.

Results

Brain AVT: Effects of Body Length, Lake, and Season

Table 2 summarizes the data on brain AVT content by lake, fish size, and season. In our first regression model, we attempted to relate the log brain AVT to body length while factoring in two categorical variables: lake (i.e., differences among the seven study lakes) and season (differences in brain AVT among fish collected in spring, summer, and fall). In this model, we were unable to test the lake × season interaction because three of the study lakes were only sampled during one season. The model (Eq. 1) obtained following the exclusion of the lake term (which failed to enter the model, $p < 0.05$) is presented below:

ANCOVA 1: log AVT versus length, lake × length, season:

$$\text{(1) log brain AVT} = 1.849 + \left\{ \left[1.167 + \begin{pmatrix} 0.642 \text{ Welly} \\ 0.501 \text{ Brochard} \\ 0.221 \text{ Pyrolle} \\ 0.017 \text{ de la Loutre} \\ 0.000 \text{ Franciscain} \\ -0.207 \text{ Demarest} \\ -1.269 \text{ Petit Penang} \end{pmatrix} \right] \times \text{length (m)} \right\} + \begin{pmatrix} -0.087 \text{ spring} \\ 0.00 \text{ fall} \\ 0.108 \text{ summer} \end{pmatrix}$$

where $N = 291$, $R^2 = 0.56$, $SE_{est} = 0.15$, and F-ratio = 86.4 ($p < 0.001$) for length, 9.52 ($p < 0.001$) for lake × length, and 26.6 ($p < 0.001$) for season effects. Lake effect not significant. Post hoc contrasts shown by vertical lines; nonsignificant ($p < 0.05$) differences connected by vertical lines.

Except for Lake Petit Penang fish, the most significant determinant of AVT levels in the brains of these brook trout was body size (length); the relationship between brain AVT and length varied significantly across the study lakes. Thus the lake term makes a highly significant contribution in the form of the lake × length interaction. The effect of this interaction can be seen in Fig. 2 in which the AVT levels in large brook trout vary greatly among the study lakes whereas those in small brook trout do not. Thus the brain AVT – body length slopes diverge in different lakes with increasing brook trout size. The seasonal pattern of brain AVT in the seven lakes was also highly significant, with summer levels highest and spring levels lowest. This can be seen from the season term of the ANCOVA model (Eq. 1) above and from Fig. 3 which shows the seasonal average AVT levels in the brook trout from the four lakes that were studied during all three seasons.

Brain AVT in Fish from Acid and Neutral Lakes

In Eq. 1, the lake × length interaction (a measure of the brain AVT – body length slope) was highest for the most acid lakes

TABLE 2. Brain AVT levels in brook trout from softwater Laurentian lakes sampled during 1988–90. Spring fish were taken during May – June 15, summer fish during June 15 – August 25, and fall fish during August 25 – September 20. Small fish represent the average of all fish <150 mm, medium fish 150–250 mm, and large fish the average for all fish >250 mm.

	Length (mm)	*N*	Brain AVT (pg/mg) geometric mean	LOG AVT (pg/mg)	SE
			Acid pH lakes (pH ≤ 5.5)		
Lake Welly					
Spring					
Small fish	94	9	85.1	1.93	0.09
Medium fish	207	6	151.4	2.18	0.11
Large fish	300	10	204.2	2.31	0.05
Lake Brochard					
Fall					
Large fish	287	14	208.9	2.32	0.03
Lake Pyrolle					
Spring					
Small fish	130	1	74.1	1.87	
Medium fish	205	11	102.3	2.01	0.04
Large fish	316	11	162.2	2.21	0.05
Summer					
Medium fish	207	3	144.5	2.16	0.04
Large fish	336	8	331.1	2.52	0.11
Fall					
Medium fish	220	13	125.9	2.10	0.03
			Neutral pH lakes (pH > 5.5)		
Lake Demarest					
Spring					
Small fish	62	2	56.2	1.75	0.05
Medium fish	205	19	95.5	1.98	0.03
Large fish	301	7	128.8	2.11	0.03
Summer					
Small fish	120	5	102.3	2.01	0.14
Medium fish	181	9	131.8	2.12	0.07
Fall					
Medium fish	197	12	107.2	2.03	0.04
Large fish	292	3	107.2	2.03	0.02
Lake de la Loutre					
Spring					
Small fish	53	15	69.2	1.84	0.06
Medium fish	225	2	93.3	1.97	0.04
Large fish	288	16	123.0	2.09	0.04
Summer					
Medium fish	220	3	154.9	2.19	0.09
Large fish	311	8	234.4	2.37	0.04
Fall					
Medium fish	220	7	151.4	2.18	0.04
Large fish	292	7	128.8	2.11	0.04
Lake Petit Penang					
Fall					
Small fish	95	20	69.2	1.84	0.04
Medium fish	190	6	66.0	1.82	0.05
Large fish	255	1	61.6	1.79	
Lake Franciscain					
Spring					
Small fish	74	25	69.2	1.84	0.04
Medium fish	200	14	109.6	2.04	0.03
Large fish	265	9	141.3	2.15	0.05
Summer					
Small fish	140	3	151.4	2.18	0.05
Medium fish	172	12	151.4	2.18	0.05
Large fish	305	2	138.0	2.14	0.12
Fall					
Medium fish	227	9	154.9	2.19	0.06
Large fish	280	6	134.9	2.13	0.06

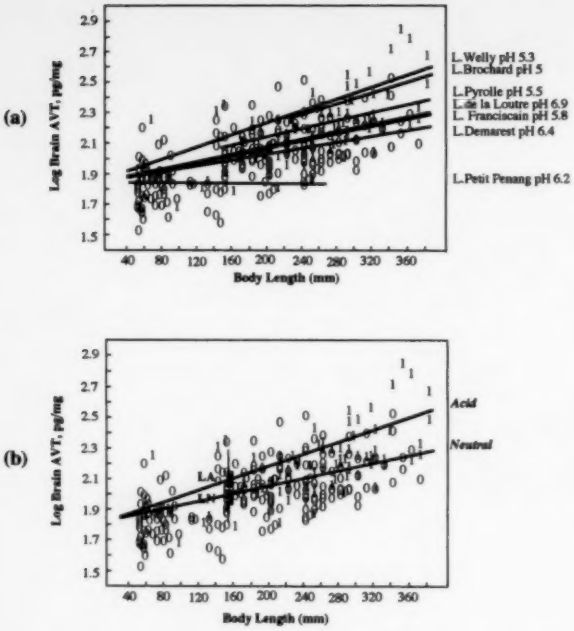


FIG. 2. (a) Size dependence of brain AVT levels in brook trout from the study lakes. Lakes with the lowest pH levels have the steepest allometric slopes. The regression lines plotted are obtained from Eq. 1, seasonally adjusted to fall levels. Numbers (0, 1) represent individual fish sampled. (b) Comparison of the size dependence of brain AVT (log pg AVT/mg brain) in brook trout from acid (pH ≤ 5.5) and neutral (pH > 5.5) lakes. Large fish from acid lakes have significantly higher AVT levels than large fish from neutral lakes. No difference in brain AVT can be detected in very small fish, as shown by the common intercept of the two regression lines (acid and neutral) obtained from Eq. 2, seasonally adjusted to fall levels. Data from our laboratory experiment (Hontela et al. 1991) are plotted to demonstrate their similar brain AVT levels to wild fish of comparable size; bars designated LA and LN represent standard errors (thick bars) and standard deviations (thin bars). LA fish were kept at pH 5.6 for 1 wk, and LN fish were kept at pH 7.0.

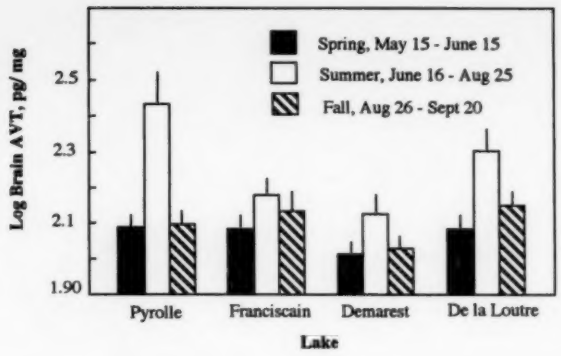


FIG. 3. Brain AVT (log pg AVT/mg brain, mean ± SEM) in brook trout sampled in Lakes Pyrolle, Franciscain, Demarest, and de la Loutre in spring, summer, and fall. The between-lake differences in the seasonal pattern of brain AVT are not significant after adjustment for body size (i.e., the lake × season interaction term made no significant contribution to Eq. 3).

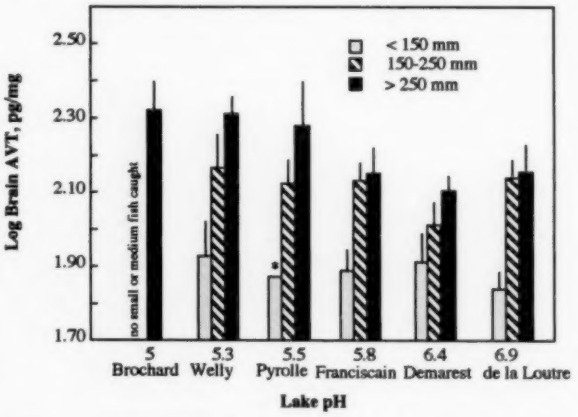


FIG. 4. Pattern of brain AVT (mean ± SEM) in small and large brook trout in relation to lake pH. Data from Lake Petit Penang are not included because this lake is an outlier in several respects (see text). *No error bar, since only one fish <150 mm was caught in Lake Pyrolle.

(Brochard and Welly) and low for the more neutral lakes. This pattern is also shown in Fig. 4 in which mean brain AVT levels of fish >150 mm were higher in acid than in neutral lakes; however, no differences could be seen for small fish. When the lakes were divided into two pH categories, acid lakes (≤pH 5.5) and neutral lakes (pH > 5.5), and the categorical variable lake pH was tested along the variables in Eq. 1, lake effects no longer entered the model. As shown in Eq. 2, the lake pH × length interaction replaced the lake × length interaction from Eq. 1:

ANCOVA 2: log AVT versus length, lake pH (pH > 5.5 = 0, pH < 5.5 = 1) × length, season:

$$(2)\quad \log \text{brain AVT} = 1.814 + \left\{ \left[1.263 + \begin{pmatrix} 0.431 \text{ acid} \\ 0.00 \text{ neutral} \end{pmatrix} \right] \times \text{length (m)} \right\} + \begin{pmatrix} -0.068 \text{ spring} \\ 0.000 \text{ fall} \\ 0.115 \text{ summer} \end{pmatrix}$$

where $N = 291$, $R^2 = 0.50$, $SE_{est} = 0.17$, and F-ratio = 93.7 ($p < 0.001$) for length, 20.7 ($p < 0.001$) for pH × length, and 24.1 ($p < 0.001$) for season effects. Lake effect not significant.

Notwithstanding the drop in R^2 from 0.56 to 0.50, the displacement of lake × lake pH, as well as the pattern in Fig. 4, suggests that most of the between-lake variability was related to

lake pH. The greater slope of log brain AVT versus body length in fish from acid lakes than in fish from more neutral lakes (Fig. 2) demonstrates this concept. Thus, large fish from acidic lakes have higher AVT levels than large fish from neutral lakes. The levels in the small fish are not different. The lake pH effect enters the model only through its effect on the slopes of the brain AVT versus length relationship and not through effects on the intercept (Fig. 2).

Not all of the significant between-lake differences in the effect of fish length (Eq. 1) can be explained by differences in lake pH. Lake Petit Penang (pH 6.2), in fact, was significantly different from all other lakes, including the other neutral pH lakes. Although AVT levels in small fish (<150 mm) from Lake Petit Penang were no different from those in other lakes (in fact, small fish from all lakes had similar AVT levels; Table 2; Fig. 4), the allometric trend for increasing brain AVT with body size was not detected in this lake. Thus the lake × length interaction term for this lake in Eq. 1 is much lower than in the other lakes and not significantly different from zero.

Seasonal Pattern of Brain AVT Levels: Is It General or Lake Specific?

While seasonal variation of brain AVT levels was not studied in much detail, it was considered important to establish that this variation could at least be handled well enough to permit seasonally adjusted comparisons among lakes. Thus the categorical variable season will only provide a useful basis for comparison if neither of the other two important effects, that is length and between-lake differences (lake), interacts with season. If none of these interactions was significant, we could then conclude that the seasonal pattern was similar among lakes.

We could see no reason to suspect that the brain AVT versus body length relationship changed with season because the season × length interaction made no significant contribution to either Eq. 1 or 2. However, the season × lake effects could not be tested in these models because three of the lakes were sampled only once (Tables 1 and 2). The original four lakes that we studied (i.e., Pyrolle, Franciscain, de la Loutre, and Demarest) were sampled during all three seasons over a 2-yr period. When the analysis was restricted to these four lakes, we obtained the following:

ANCOVA 3: log AVT versus length, lake × length, season, season × lake:

$$\text{(3)}\quad \log \text{brain AVT} = 1.852 + \left\{ \left[1.162 + \begin{pmatrix} 0.000 \text{ Pyrolle} \\ -0.022 \text{ de la Loutre} \\ -0.091 \text{ Franciscain} \\ -0.244 \text{ Demarest} \end{pmatrix} \right] \times \text{length (m)} \right\} + \begin{pmatrix} -0.032 \text{ spring} \\ 0.000 \text{ fall} \\ 0.078 \text{ summer} \end{pmatrix}$$

where $N = 171$, $R^2 = 0.38$, $SE_{est} = 0.20$, and F-ratio = 37.99 ($p < 0.001$) for length, 4.59 ($p < 0.01$) for lake × length, and 22.04 ($p < 0.001$) for season effects. Lake and lake × season effects not significant ($p < 0.05$). Post hoc contrasts shown by vertical lines; nonsignificant ($p < 0.05$) differences connected by vertical lines.

While this model explained only 38% of the variance in log brain AVT (compared with 56 and 50% in Eq. 1 and 2), this is an understandable result of dropping the two most acid lakes, as well as Lake Petit Penang, resulting in a substantial reduction in the AVT variance. However, the F-value for the effect of season in Eq. 3 is highly significant ($p < 0.001$) and similar to that in Eq. 1 and 2. Thus, the inability of the season × lake interaction to enter the model strongly supports the picture conveyed by Fig. 3 that the seasonal pattern (low values in spring, highest in summer, followed by medium values in fall) did not vary between lakes.

Discussion

Changes in the secretion of osmoregulatory hormones reflect homeostatic efforts of fish challenged by osmoionic stressors in their environment. Brain AVT increases in juvenile brook trout subjected to sublethal acid conditions in the laboratory (Hontela et al. 1991), and the aim of the present study was to quantify the interactions between pH, season, size of the fish, and the AVT response in the field. This is the first study to establish the patterns of variation of brain AVT in brook trout from lakes ranging in pH from acidic to neutral and to test the potential of AVT as an endocrine biomarker of acid stress. It is a first attempt to field calibrate, by means of multivariate analyses (ANCOVA), an endocrine parameter for use in an ecotoxicological study. This multidisciplinary approach (involving in our study endocrinology, ecology, limnology, biostatistics, and toxicology) has been strongly advocated by recent reviews in environmental toxicology (e.g., Kendall and Lacher 1991).

Choosing the Appropriate Lakes to Study Sublethal Acid Stress in Brook Trout

The choice of appropriate lakes for investigating sublethal acid stress in fish is difficult because so little is known about the effects of mild osmoionic disturbances in fish populations. Most laboratory studies on acid stress in brook trout have focussed on pH's <5.0 at which osmoionic effects are severe and direct lethality is common. Exposure of brook trout to sublethal pH (e.g., pH 5.2) in the laboratory may cause relatively small changes in plasma Na^+ concentrations, yet it does modify Na^+ fluxes across the gills (McDonald and Milligan 1988; Wood et al. 1988a, 1988b). It is precisely during exposure to such sublethal pH regimens that osmoregulatory hormones, the effectors of osmoionic homeostasis, may be particularly useful as early-warning indicators of acid stress.

To increase our likelihood of encountering sublethally acid-stressed populations, we chose to work in an area that receives acid rain and exhibits a wide range in buffering capacity (Boulet and Jacques 1987; Dupont 1989). To test, in the field, the sensitivity of brain AVT as an early-warning indicator of acid stress, we originally (September 1988–89) selected four lakes representing a range of pH from pH 5.5 to near-neutral pH 6.9. However, it was not until we extended our pH range downward to 5.0 by sampling Lakes Welly (pH 5.3) and Brochard (pH 5.0) that we could confidently associate variations in brain AVT levels with lake acidity. Although none of the fish we sampled showed excess mucus in the gills or swollen secondary lamellae, the gill pathology of severely acid-stressed fish (Chevalier et al. 1985; Karlsson-Norrgren et al. 1986; Chevalier et al. 1985; Walker et al. 1988), we caught few or no parr in the three most acidic lakes (Table 1). This suggests that the long-term physiological performance of the fish may be adversely affected by chronic exposure to pH 5.0–5.5, the pH range at which we observed the strongest relationship between brain AVT and pH (Fig. 2).

Although laboratory studies have shown that the adverse effects of low pH are exacerbated by low Ca^{2+} (<0.5 mg·L) and high monomeric aluminum (>300 μg·L) in the water (Wood et al. 1988a, 1988b), we were unable to statistically isolate the contribution of calcium and aluminum to the patterns of brain AVT because they covary too closely with pH across the lakes in our study. This close linkage between metal cations and pH is of course inevitable in any field study carried out in acid-sensitive regions, since the main geochemical determinants of calcium and aluminum in such waters are ion-exchange reactions between clay minerals and H^+ (Harvey et al. 1981). Notwithstanding the close relationship of calcium and aluminum to pH, we do not consider it likely that these two factors have been contributing significantly to the patterns of brain AVT described in this study because our estimates of aluminum concentrations in water are uniformly low (total Al <300 μg/L, monomeric Al <75 μg/L), even in our most acid lakes, and are thus outside the range considered to be toxic. The calcium levels in our lakes (1.3–2.5 mg/L) are within the range considered medium for brook trout by Wood et al. (1988a, 1988b).

Comparing Laboratory Experiments to Between-Lake Comparisons Using ANCOVA Modelling

The increase in brain AVT with decreasing pH in the pH 5–6 range corroborates our earlier laboratory study on hatchery-reared juvenile brook trout (Hontela et al. 1991), which showed that brain AVT concentrations were elevated after a 1-wk exposure to mildly acidic conditions (pH 5.6). Besides demonstrating that brain AVT levels are elevated in fish exposed to sublethal acid pH, our ANCOVA models showed that the response is greater in large brook trout than in smaller ones and is undetectable in fish <150 mm. Brain AVT concentrations in young-of-the year fish exhibited no significant between-lake variation (Fig. 2). To further complicate matters, the analyses consistently showed a strong positive relationship between brain AVT levels and body size (Fig. 2). The strong allometry of brain AVT shown in the field study and the dependence of the AVT – body length slope on pH (Fig. 2) detected by the ANCOVA analyses would not have been revealed in laboratory studies that generally use fish of a similar size.

The field studies also showed a seasonal pattern in brain AVT. This seasonal pattern was very similar in the four lakes that we sampled across seasons (the lake × season interaction term was very weak and not statistically significant; Eq. 3) and did not reflect seasonal changes in lake pH. Hormone concentrations in fish often vary seasonally (Hontela and Stacey 1990), and photoperiod, temperature, and food availability are only some of the factors that may modulate the hormonal response to a particular environmental variable, such as the acidity of the water. Resistance to acidity has been shown to increase with decreasing temperature in rainbow trout (*Oncorhynchus mykiss*) (Robinson et al. 1976), and the protective effect of calcium is well documented (Wood et al. 1988a, 1988b). However, little is known about seasonal variation in AVT levels in fish. Temperature does affect brain AVT concentrations in goldfish (*Carassius auratus*) kept under controlled temperature regimens in the laboratory (A. Hontela, unpublished data). The effects of other parameters such as reproductive status or water characteristics other than pH are presently unknown. One environmental parameter that has attracted our attention in the present study is the colour of the water. A large number of lakes in the acid-sensitive region of the Canadian Shield are brown, yet the modulating influences of humic material on the endocrine response of fish to acid pH has not yet been investigated. Brain AVT levels in fish from Lake Petit Penang, a very brown lake (Table 1), were much lower than the AVT levels in fish from other nonacid lakes, and the allometric trend for increasing brain AVT concentrations with body size was not detected. The reasons for this lake being an outlier may be related in some way to its high humic content. A protective effect of humic material on survival of fish has been reported in acid waters (Karlsson-Norrgren et al. 1986).

The regression model has enabled us to adjust the AVT levels for the effect of season because the seasonal variation was similar in the four lakes sampled in spring, summer, and fall. The AVT levels were highest in the summer, intermediate in the fall, and lowest in the spring in all four lakes (two neutral lakes and two mildly acidic lakes). The seasonally fluctuating environmental or physiological factors that may influence the AVT secretion during an exposure to acid pH are presently being investigated.

Physiological Role of AVT: Effects on Blood Flow and Permeability

The physiological role of AVT is that of a hormonal mediator of osmoionic homeostasis, through its vasoconstrictor action on the gill capillaries, reduction of gill permeability, and increased diuresis (Lahlou and Giordan 1970; Bennett and Rankin 1986). These responses could counteract the ion loss and the dilution of body fluids, the physiological responses characteristic of acid stress (Audet et al. 1988; Wood 1988a, 1988b). The increased brain AVT in larger fish subjected to acid water may indicate an activation of homeostatic mechanisms aimed at reducing the acid stress. It has already been shown that older fish are more resistant to the detrimental effects of exposure to acid water than are fish in the earlier stages of development (Baker and Schofield 1980; Cleveland et al. 1986; Gunn and Noakes 1987). Robinson et al. (1976) reported that larger rainbow trout had better survivals in acid water than smaller fish. Our data on brain AVT suggest that fish in earlier stages of development may be less resistant to acid water because they do not sufficiently activate their osmoionic homeostatic mechanisms, as evidenced by their inability to increase the brain AVT concentrations during exposure to acid pH. We are presently testing this hypothesis.

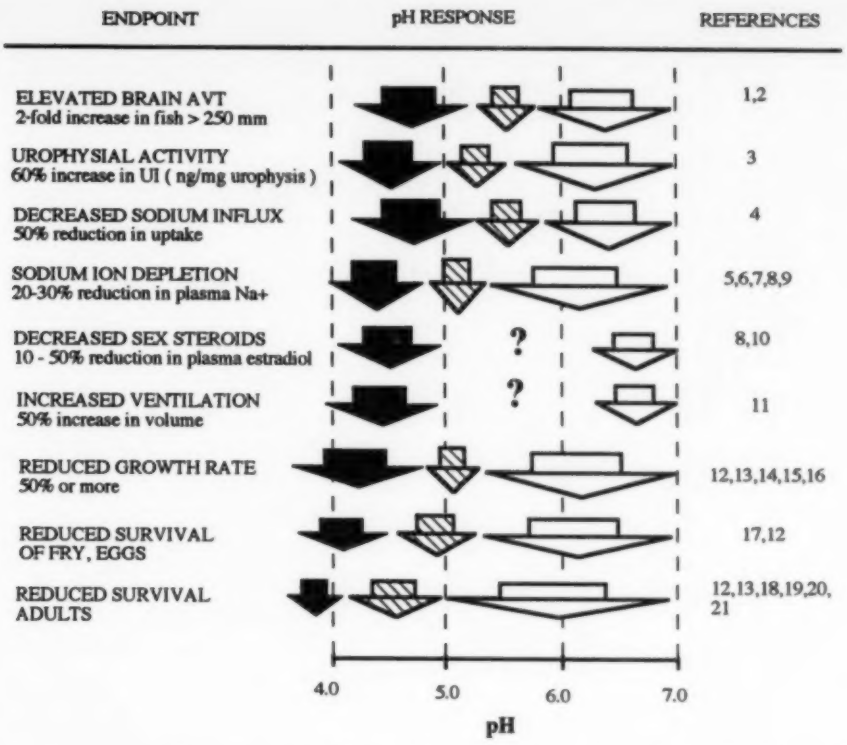


FIG. 5. Comparison of sensitivities of brain AVT and various acid-stress endpoints in brook trout based on the following published studies: 1, present study; 2, Hontela et al. 1991; 3, Hontela et al. 1989; 4, McDonald and Milligan 1988; 5, Wood et al. 1988a; 6, Wood et al. 1988b; 7, Fryer et al. 1988; 8, Mount et al. 1988a; 9, Booth et al. 1988; 10, Tam et al. 1987; 11, Walker et al. 1988; 12, Menendez 1976; 13, Siddens et al. 1986; 14, Mount et al. 1988b; 15, Rodgers 1984; 16, Tam et al. 1988; 17, Cleveland et al. 1986; 18, Beggs and Gunn 1986; 20, Robinson et al. 1976; 21, Schofield and Trojnar 1980. Only data obtained from exposures to low calcium (<2 mg/L) and low aluminum (<300 μg/L) were included. Solid arrow, endpoint fully manifested; hatched arrow, partially or inconsistently manifested; open arrow, no effect. Arrow width denotes relevant pH range.

Brain AVT as a Potential Biomarker for Sublethal Acid Stress

The comparative study using ANCOVA, which adjusted for the effect of body size and season, allowed us to detect a relationship between brain AVT and the pH of the study lakes (Fig. 4). Thus, knowing the pH of the lake, the season, and the size of the fish, we can now make at least crude predictions of the brain AVT levels in the fish. Brain AVT thus seems to offer some potential as a hormonal bioindicator of acid stress in fish. Species and strain differences in acid tolerance of fish have been reported (Robinson et al. 1976) and these differences could be partly related to differing dynamics of AVT secretion during exposure to acid pH. Future studies must further validate the AVT response as an endocrine bioindicator of acid stress in fish by comparing the AVT response in acid-resistant and acid-tolerant species.

This field study corroborated our previous laboratory study and provided further support for brain AVT as a potential biomarker for acid stress in brook trout. Elevated brain AVT levels in response to acid were detectable at pH 5.6 in our laboratory study (Hontela et al. 1991) and in brook trout from all lakes below pH 5.5 (Fig. 4). Another endocrine response, elevated levels of the osmoregulatory hormone urotensin I in the urophysis, was shown in a laboratory study (Hontela et al. 1989) to be activated at about the same pH level as the increase in AVT (Fig. 5). The only blood osmotic indicator that appears to be as sensitive as the osmoregulatory hormonal response is the decrease in Na^+ influx, which becomes detectable at about the same level of sublethal acid stress (McDonald and Milligan 1988) (Fig. 5). However, the rate of ion influx has never been measured on feral fish and will likely not prove to be a practical biomarker for field use, since it involves the use of the radiotracer ^{22}Na. Although reduced sodium influx is a highly sensitive endpoint, the more easily measured sodium depletion of plasma does not become detectable until around pH 5.2, which is about the same pH at which reduced survival of eggs and fry is observed (Fig. 5). Figure 5 presents a summary comparison of the sensitivies of the pH responses for a number of physiological and population endpoints that have been related to acid stress in field and laboratory studies on brook trout.

Our study also points out a number of complications that may, upon further study, prove to be relevant to field biomarkers of sublethal toxic stress in general. In our laboratory study on AVT in juvenile brook trout, we found that the total range of variability

exhibited by these fish was about twofold. The present study, on the other hand, showed that wild fish exhibit variability of about an order of magnitude (50–500 pg AVT/mg). The portion of this variability that could be attributed to between-lake differences in pH was only about twofold and depended greatly on the size of the fish. Fish size and seasonal factors both explained a larger fraction of the observed variability than did pH, and the unexplained variation (residual) is itself about twofold. This presents a complication for any field biomarker. The meaningful (or indicative) portion of the field variability (i.e. the signal), is small relative to the total spectrum of variability encountered and difficult to discriminate from residual noise. Few ecotoxicological biomarkers have been subjected to rigorous field testing and statistical evaluation, and for the majority, little is known about the types of mitigating influences that might limit their interpretability or complicate their use. The signal-to-noise properties that a biomarker displays under field situations will likely be very different from those displayed under controlled laboratory conditions, and this situation may greatly hinder the interpretation of the biomarker responses and limit their use in ecotoxicological studies.

Acknowledgements

This study was funded by a FODAR-UQAM grant to G.C. and A.H. and a Natural Sciences and Engineering Research Council of Canada grant to J.B.R. The help of D. Rowan, S. Kesting, S. Lamontagne, and B. Libman in collecting the fish is gratefully acknowledged. We thank the Direction de MLCP (Trois-Rivières), Gouvernement du Québec, for their support of our research in the Réserve Faunique Mastigouche and V. Guillemette for facilitating our sampling. Special thanks to J. Archambault (MLCP, Shawinigan) for making available to us the physicochemical data on Mastigouche lakes and his expertise.

References

AUDET, C., R.S. MUNGER, AND C.M. WOOD. 1988. Long-term sublethal acid exposure in rainbow trout (*Salmo gairdneri*) in soft water: effects on ion exchange and blood chemistry. Can. J. Fish. Aquat. Sci. 45: 1387–1398.

BAKER, J.P., AND C.L. SCHOFIELD. 1980. Aluminum toxicity to fish as related to acid precipitation and Adirondack surface water quality, p. 292–293. *In* Proc. Int. Conf. Ecol. Impact Acid Precip. Norway 1980, SNSF Project Oslo, Norway.

BEGGS, G.L., AND J.M. GUNN. 1986. Response of lake trout (*Salvelinus namaycush*) and brook trout (*Salvelinus fontinalis*) to surface water acidification in Ontario. Water Air Soil Pollut. 30: 711–717.

BENNETT, M.B., AND J.C. RANKIN. 1986. The effects of neurohypohysial hormones on the vascular resistance of the isolated perfused gill of the euroean eel, *Anguilla anguilla*. Gen. Comp. Endocrinol. 64: 60–66.

BOOTH, C.E., D.G. MCDONALD, B.P. SIMONS, AND C.M. WOOD. 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1563–1574.

BOULET, G., AND G. JACQUES. 1987. Sommaire des données de la qualité des eaux de précipitations, Précipitations acides QEN/PA-37.

BROWN, S.B., J.G. EALES, R.E. EVANS, AND T.J. HARA. 1984. Interrenal, thyroidal, and carbohydrate responses of rainbow trout (*Salmo gairdneri*) to environmental acidification. Can. J. Fish. Aquat. Sci. 41: 36–45.

BROWN, S.B., R.E. EVANS, H.S. MAJEWSKI, G.B. SANGALANG, AND J.F. KLAVERKAMP. 1990. Responses of plasma electrolytes, thyroid hormones, and gill histology in Atlantic salmon (*Salmo salar*) to acid and limed river waters. Can. J. Fish. Aquat. Sci. 47: 2431–2440.

CHEVALIER, G., L. GAUTHIER, R. LIN, R.S. NISHIOKA, AND H. BERN. 1986. Effect of chronic exposure to an acidifed environment on the urophysis of the brook trout, *Salvelinus fontinalis*. Exp. Biol. 45: 291–299.

CHEVALIER, G., L. GAUTHIER, AND G. MOREAU. 1985. Histopathological and electron microscopic studies of gills of brook trout, *Salvelinus fontinalis*, in acidified lakes. Can. J. Zool. 63: 2062–2070.

CLEVELAND, L., E.E. LITTLE, S.J. HAMILTON, D.R. BUCKLER, AND J.B. HUNN. 1986. Interactive toxicity of aluminum and acidity to early life stages of brook trout. Trans. Am. Fish. Soc. 115: 610–620.

DUPONT, J. 1989. État de l'acidité des lacs de la région de la Mauricie. Publication QEN/PA-33/1: 119 p.

FRENETTE, J., AND J.J. DODSON. 1984. Brook trout (*Salvelinus fontinalis*) population structure in acidified lac Tantaré, Québec. Can. J. Fish. Aquat. Sci. 41: 865–877.

FRYER, J.N., W.H. TAM, B. VALENTINE, AND R.E. TIKKALA. 1988. Prolactin cell cytology, plasma electrolytes, and whole-body sodium efflux in acid-stressed brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1212–1221.

GUNN, J.M., AND D.L.G. NOAKES. 1987. Latent effects of pulse exposure to aluminum and low pH on size, ionic composition, and feeding efficiency of lake trout (*Salvelinus namaycush*) alevins. Can. J. Fish. Aquat. Sci. 44: 1418–1424.

HARVEY, H.H., R.C. PIERCE, P.J. DILLON, J.R. KRAMER, AND D.M. WHELPDALE. 1981. Acidification in the Canadian aquatic environment: scientific criteria for assessing the effects of acidic deposition on aquatic ecosystems. NRCC Associate Committee on Scientific Criteria for Environmental Quality, Subcommittee on Water, Ottawa, Ont.

HONTELA, A., J.B. RASMUSSEN, D. KO, L. LEDERIS, AND G. CHEVALIER. 1991. Arginine vasotocin, an osmoregulatory hormone, as a potential indicator of acid stress in fish. Can. J. Fish. Aquat. Sci. 48: 256–263.

HONTELA, A., Y. ROY, R. VAN COILLIE, K. LEDERIS, AND G. CHEVALIER. 1989. Differential effects of low pH and aluminium on the caudal neurosecretory system of the brook trout, *Salvelinus fontinalis*. J. Fish Biol. 35: 265– 273.

HONTELA, A., AND N.E. STACEY. 1990. Regulation of reproductive cycles in Cyprinidae, p. 53–77. *In* A.D. Munro, A.P. Scott, and T.J. Lam [ed.] Reproductive seasonality in teleosts: environmental influences. CRC Press, Boca Raton, Fla.

HUGGETT, R.J., R.A. KIMERLE, P.M. MEHRLE JR., AND H.L. BERGMAN. 1992. Biomarkers: biochemical, physiological, and histological markers of anthropogenic stress. SETAC Special Publication Series. Lewis Publishers, Inc., Boca Raton, Fla. 347 p.

KARLSSON-NORRGREN, L., W. DICKSON, O. LJUNGBERG, AND P. RUNN. 1986. Acid water and aluminum exposure: gill lesions and aluminium accumulation in farmed brown trout, *Salmo trutta* L. J. Fish Dis. 9: 1–9.

KENDALL, R.J., AND T.E. LACHER. 1991. Ecological modeling, population ecology, and wildlife toxicology: a team approach to environmental toxicology. Environ. Toxicol. Chem. 10: 297–299.

LACROIX, G.L., D.J. GORDON, AND D.J. JOHNSTON. 1985. Effects of low environmental pH on the survival, growth, and ionic composition of post-emergent Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 42: 768–775.

LACROIX, G.L., AND D.R. TOWNSEND. 1987. Responses of juvenile Atlantic salmon (*Salmo salar*) to episodic increases in acidity of Nova Scotia rivers. Can. J. Fish. Aquat. Sci. 44: 1475–1484.

LAHLOU, B., AND A. GIORDAN. 1970. Le contrôle hormonal des échanges et de la balance de l'eau chez le Teleostéen d'eau douce *Carassius auratus* intact et hypophysectomisé. Gen. Comp. Endocrinol. 14: 491–509.

LAPPALAINEN, A., M. RASK, AND P.J. VUORINEN. 1988. Acidification affects the perch, *Perca fluviatilis*, populations in small lakes of southern Finland. Environ. Biol. Fishes 21: 231–239.

LEUVEN, R.S.E.W., AND F.G.F. PYEN. 1987. Impact of acidification and eutrophication on the distribution of fish species in shallow and lentic soft waters of The Netherlands: an historical perspective. J. Fish Biol. 31: 753–774.

LYONS, J. 1982. Effects of lethal acidity on plasma sodium concentrations in yellow perch (*Perca flavescens*) from a naturally acidic and a naturally alkaline lake. Comp. Biochem. Physiol. 73A: 437–440.

MAETZ, J., J. BOURGUOT, B. LAHLOU, AND J. HOUDRY. 1964. Peptides neurohypophysaires et osmoregulation chez *Carassius auratus*. Gen. Comp. Endocrinol. 4: 508–522.

MALTE, H. 1986. Effects of aluminium in hard acid water on metabolic rate, blood gas tensions and ionic status in rainbow trout. J. Fish Biol. 29: 187–198.

MCDONALD, D.G., AND C.L. MILLIGAN. 1988. Sodium transport in the brook trout, *Salvelinus fontinalis*: effects of prolonged low pH exposure in the presence and absence of aluminum. Can. J. Fish. Aquat. Sci. 45: 1606–1613.

MENENDEZ, R. 1976. Chronic effects of reduced pH on brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 33: 118–123.

MOUNT, D.R., J.R. HOCKETT, AND W.A. GERN. 1988a. Effect of long-term exposure to acid, aluminum, and low calcium on adult brook trout (*Salvelinus fontinalis*). 2. Vitellogenesis and osmoregulation. Can. J. Fish. Aquat. Sci. 45: 1633–1642.

MOUNT, D.R., C.G. INGERSOLL, D.D. GULLEY, J.D. FERNANDEZ, T.W. LAPOINT, AND H.L. BERGMAN. 1988b. Effect of long-term exposure to acid, aluminum, and low calcium on adult brook trout (*Salvelinus fontinalis*). 1. Survival, growth, fecundity, and progeny survival. Can. J. Fish. Aquat. Sci. 45: 1623–1632.

RASMUSSEN, J.B., L. GODBOUT, AND M. SCHALLENBERG. 1989. The humic content of lakewater and its relationship to watershed and lake morphometry. Limnol. Oceanogr. 34: 1336–1343.

RASMUSSEN, J.B., D.J. ROWAN, D.R.S. LEAN, AND J.H. CAREY. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Can. J. Fish. Aquat. Sci. 47: 2030–2038.

ROBINSON, G.D., W.A. DUNSON, J.E. WRIGHT, AND G.E. MAMOLITO. 1976. Differences in low pH tolerance among strains of brook trout (*Salvelinus fontinalis*). J. Fish Biol. 8: 5–17.

RODGERS, D.W. 1984. Ambient pH and calcium concentrations as modifiers of growth and calcium dynamics of brook trout, *Salvelinus fontinalis*. Can. J. Fish. Aquat. Sci. 41: 1774–1780.

ROWAN, D.J., AND J.B. RASMUSSEN. 1992. Why don't Great Lakes fish reflect environmental concentrations of organic contaminants: an analysis of between-lake variability in ecological partitioning of PCBs and DDT. J. Great Lakes Res. 18: 724–741.

SAWYER, W.H. 1977. Evolution of neurohypophyseal hormones and their receptors. Fed. Proc. 36: 1842–1847.

SCHOFIELD, C.L., AND J.R. TROJNAR. 1980. Aluminum toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters, p. 341–363. *In* T.Y. Toribara, M.W. Miller, and P.E. Morrow [ed.] Polluted rain. Plenum Press, New York, N.Y.

SIDDENS, L.K., W.K. SEIM AND L.R. CURTIS. 1986. Comparison of continuous and episodic exposure to acidic, aluminum-contaminated waters of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 43: 2036–2040.

TAM, W.H., L. BIRKETT, R. MAKARAN, P.D. PAYSON, D.K. WHITNEY, AND C.K.-C. YU. 1987. Modification of carbohydrate metabolism and liver vitellogenic function in brook trout (*Salvelinus fontinalis*) by exposure to low pH. Can. J. Fish. Aquat. Sci. 44: 630–635.

TAM, W.H., J.N. FRYER, I. ALI, M.R. DALLAIRE, AND B. VALENTINE. 1988. Growth inhibition, gluconeogenesis, and morphometric studies of the pituitary and interrenal cells of acid-stressed brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1197–1211.

US EPA. 1986. Characteristics of lakes in the eastern United States. Vol. 3. Data compendium of site characteristics and chemical variables. US EPA, Washington, D.C. 439 p.

WALKER, R.L., C.M. WOOD, AND H.L. BERGMAN. 1988. Effects of low pH and aluminium on ventilation in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1614–1622.

WATT, W.D., C.D. SCOTT, AND W.J. WHITE. 1983. Evidence of acidification of some Nova Scotian rivers and its impact on Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. 40: 462–473.

WENDELAAR BONGA, S.E., P.H.M. BALM, AND G. FLIK. 1988. Control of prolactin secretion in the teleost *Oreochromis mossambicus*: effects of water acidification. Gen. Comp. Endocrinol. 72: 1–12.

WILKINSON, L. 1989. SYSTAT: the system for statistics. Systat Inc., Evanston, Ill.

WOOD, C.M., D.G. MCDONALD, C.E. BOOTH, B.P. SIMONS, C.G. INGERSOLL, AND H.L. BERGMAN. 1988a. Physiological evidence of acclimation to acid aluminum stress in adult brook trout (*Salvelinus fontinalis*). 1. Blood composition and net sodium fluxes. Can. J. Fish Aquat. Sci. 45: 1587–1596.

WOOD, C.M., R.C. PLAYLE, B.P. SIMONS, G.G. GOSS, AND D.G. MCDONALD. 1988b. Blood gases, acid–base status, ions and hematology in adult brook trout (*Salvelinus fontinalis*) under acid/aluminum exposure. Can. J. Fish. Aquat. Sci. 45: 1575–1586.

Iron Budgets in Temperate Lakes

Gertrud K. Nürnberg

Freshwater Research, RR 1, Baysville, ON P0B 1A0, Canada

and Peter J. Dillon

Dorset Research Centre, Ontario Ministry of Environment and Energy, P.O. Box 39, Dorset, ON P0A 1E0, Canada

Nürnberg, G.K., and P.J. Dillon. 1993. Iron budgets in temperate lakes. Can. J. Fish. Aquat. Sci. 50: 1728–1737.

Iron mass balances for eight lakes (79 lake-years of data) on the Precambrian Shield were measured including catchment, atmospheric, and internal sources of iron and compared with literature data. In most cases, internal load from anoxic sediment surfaces was important. Net (R_{ext}) and gross (R_{tot}) Fe retentions were correlated with internal load and with morphometry and hydrology, respectively. An average annual settling velocity of Fe computed from the relationship of R_{tot} with the annual water load (q_s) ranged from 12 to 26 m/yr. A simple mass balance model, similar to that used successfully for phosphorus, was used to predict annual average Fe concentration. Although model predictions of average Fe concentration of the lake outflow (L_{out}/q_s) and observed concentrations (TFe) were significantly correlated, TFe was underestimated by the model in the Precambrian Shield lakes but overestimated in acid-stressed lakes in the Sudbury, Ontario region. Since the deviations were correlated with organic content of the water or sediment, dissolved organic carbon concentration (DOC), colour of the lake water, and loss on ignition of the surface sediments proved to be important secondary variables: the best predictive model was TFe = −43.9 + 0.81 L_{out}/q_s + 30.0 DOC.

Le bilan massique du fer dans huit lacs (79 années-lacs de données) du bouclier précambrien, y compris les sources internes, atmosphériques et les sources situées dans le bassin hydrographique de cet élément, est comparé, aux données publiées. Dans la plupart des cas, la charge interne provenant de la surface des sédiments anoxiques est importante. La rétention nette (R_{ext}) et brute (R_{tot}) du Fe a été corrélée à la charge interne ainsi qu'à la morphométrie et l'hydrologie, respectivement. On a calculé que la vitesse annuelle moyenne de dépôt du Fe, à partir du rapport de R_{tot} à la charge annuelle de l'eau (q_s) variait de 12 à 26 m/an. Un simple modèle du bilan des matières, semblable à celui qui a donné de bons résultats avec le phosphore, a permis de prédire la concentration moyenne annuelle du Fe. Bien que les prédictions modélisées de la concentration moyenne de Fe dans l'émissaire du lac (L_{out}/q_s) et les concentrations observées (TFe) aient été corrélées de façon significative, le paramètre TFe était sous-estimé par le modèle dans les lacs du bouclier précambrien, mais surestimé dans les lacs acidifiés de la région de Sudbury (Ontario). Comme les écarts étaient corrélés avec la teneur en matières organiques de l'eau ou des sédiments, la concentration de carbone organique dissous (COD), la couleur de l'eau lacustre et la perte au feu des sédiments de surface se sont révélées des variables secondaires importantes. Le meilleur modèle de prédiction correspondait à la formule suivante : TFe = −43,9 + 0,81 L_{out}/q_s + 30,0 COD.

Received June 9, 1992
Accepted February 16, 1993
(JB516)

Reçu le 9 juin 1992
Accepté le 16 février 1993

Iron is an essential micronutrient for phytoplankton and can control phytoplankton abundance when in short supply (Murphy and Yesaki 1983). Fe is an important element in lakes because of its chemical and biological interactions with other lake water constituents. It can control the concentration and movement of nutrients such as phosphate, major elements, and metals (e.g., cadmium, lead, cobalt, copper, zinc, and nickel) in the water column (e.g., Bacchini and Joller 1981; Sholkovitz and Copland 1982; Tessier et al. 1985).

The chemistry and adsorption properties of Fe have been studied extensively in the laboratory (e.g., Schnitzer and Kerndorff 1981; Liang and Morgan 1990) and field (Davis 1982; Urban et al. 1990), and numerous kinetic and thermodynamic models for the chemistry and physics of different Fe species in water exist (e.g., Stumm and Morgan 1981). But many of these models fall short when tried for quantitatively describing the situation in real lakes. It is often impossible to determine all the concentrations and chemical activities of the constituents involved, and the interaction with colloids may influence the behaviour of Fe drastically (Morel and Geschwend 1987).

Little is known about whole-lake and catchment fluxes. Few studies of whole-lake mass Fe balances have been done (see references in Table 2). In only one case (Dillon et al. 1988) have Fe budgets of more than one lake been reported and the five lakes in that study are quite extraordinary, receiving high inputs of mineral acid, Fe, and other metals from smelters in the Sudbury, Ontario, area.

In this study, we measured Fe mass balances of several lakes on the Canadian Precambrian Shield and pooled these data with all available quantitative information on Fe fluxes in other North American and European lakes. We present a general model using Fe budgets to predict annual Fe concentrations in a variety of lakes. In particular, we evaluated the predictive capability of a simple mass balance model similar to that used successfully for phosphorus (Vollenweider 1968; Dillon and Rigler 1975).

Many lake characteristics and the components of the mass balance model such as retention, external load, and internal load were investigated separately, since the chemistry of Fe is so complex.

For example, we determined oxygen and organic carbon

TABLE 1. Morphometric characteristics of the study lakes. The A Lakes had Fe mass balances measured; the B lakes did not. na, data not available.

Lake	A_d (ha)	A_0 (ha)	$\bar{z}$ (m)	τ_w (yr)	q_s (m/yr)
A lakes					
Blue Chalk	105.9	52.35	8.5	5.48	1.61
Chub	271.8	34.41	8.9	2.06	4.46
Crosson	521.8	56.74	9.2	1.67	5.71
Dickie	406.4	93.60	5.0	1.71	2.99
Harp	470.7	71.38	13.3	3.16	4.33
Jerry	861.0	50.10	12.4	1.25	9.99
Plastic	95.5	32.14	7.9	3.90	2.10
Red Chalk	532.4	57.13	14.2	2.63	5.57
B lakes					
Basshaunt	791.0	47.3	7.7	0.84	9.58
Bigwind	396.0	111.0	10.7	5.45	2.04
Buck	251.0	40.3	10.9	3.18	3.57
Glen	97.0	16.3	7.2	2.21	3.41
Gravenhurst Bay	790.0	179.4	9.8	3.60	2.71
Gullfeather	982.0	65.9	4.8	0.59	8.54
Little Clear	569.0	10.9	8.1	0.28	29.91
Paint-10	na	1.0	3.2	na	na
Solitaire	379.0	124.0	13.3	7.91	1.75
Walker	258.0	68.2	6.2	2.98	2.17

concentrations, since the chemical properties of Fe depend on these constituents. In the presence of oxygen and in absence of organic acids, Fe forms hydrous oxide particles that can adsorb other dissolved metals and phosphate; these particles settle to the lake bottom. The metals and phosphate remain adsorbed until oxygen is depleted and the redox potential of the medium changes. Under reducing conditions the adsorbed species are dissolved and, depending on their chemical behaviour, can be released into the anoxic water (either the pore water within the sediment or the anoxic hypolimnetic water), coprecipitate in the reduced state as sulfides, or become adsorbed on insoluble iron sulfides (Gunkel and Sztraka 1986). Incidences of coprecipitation (Sholkovitz and Copland 1982), adsorption (Tessier et al. 1985), and comigration of metals and phosphate have been described previously (Bacchini and Joller 1981). Changes in the redox state of Fe are also important in the study of acid lakes, as the reduction of Fe generates alkalinity (Cook et al. 1986; Psenner 1988). In a naturally acidic, brown-water lake, organic acid concentration is high and can control Fe concentration by increasing ferric oxide solubility (at pH > 5) or by precipitation of humates (at pH < 5, Nova Scotia lakes, Urban et al. 1990).

We hypothesized that some of these Fe fluxes depend on input and concentration of organic acids measured as dissolved organic carbon (DOC), colour, or organic content of the sediment (loss on ignition, LOI). We also determined whether Fe retention depends on lake morphometry and hydrology, as is the case for retention of other substances (e.g., phosphorus, Kirchner and Dillon 1975; nitrogen, and DOC, Dillon and Molot 1990) and determined an average settling velocity from relationships of retention with hydrological characteristics.

Throughout our analysis, we distinguished between Fe derived from external sources such as runoff from the terrestrial catchment and atmospheric deposition on the lake's surface and Fe from internal sources such as the anoxic sediment surfaces. Mass balance equations and retentions are also calculated with explicit consideration of internal Fe load to demonstrate its importance.

We have not tried to explore the chemistry and kinetics of Fe and DOC in natural waters. This has already been done in numerous studies. Instead, we present a more general overview of the possible fate of Fe in a variety of lakes. In this way, we provide a predictive tool that can be used in the management of drinking and industrial water supply where, for example, in the United States (Robinson et al. 1987) and in Australia (Khoe and Waite 1989), Fe exceeds the recommended levels in half of the resources.

Materials and Methods

Description of Symbols

$\bar{z}$	Mean depth (m)
τ_w	Water residence time (yr)
q_s	Annual water load (m/yr)
A_d	Watershed area (ha)
A_0	Lake surface area (ha)
Fe	Iron (μg/L)
TFe	Observed annual average whole lake total Fe (μg/L)
TFE_{pred}	Predicted annual average whole lake total Fe (μg/L)
L_{ext}	External Fe load ($mg \cdot m^{-2} \cdot yr^{-1}$)
L_{int}	Internal Fe load ($mg \cdot m^{-2} \cdot yr^{-1}$)
L_{tot}	Total Fe load ($mg \cdot m^{-2} \cdot yr^{-1}$)
L_{out}	Output of Fe via lake outflow ($mg \cdot m^{-2} \cdot yr^{-1}$)
R_{ext}	Fe retention based on external load
R_{tot}	Fe retention based on total load
DOC	Dissolved organic carbon (mg/L)
Colour	Average epilimnetic colour (Hazen Units, mg Pt/L)
LOI	Loss on ignition, organic content of sediment (%)

Study Lakes

Fe mass balances were measured for eight lakes in south-central Ontario (45°–45°30′N, 78°–79°W, Table 1, A lakes). Budgets were measured for Blue Chalk, Chub, Dickie, and Harp from 1978 to 1988, for Red Chalk from 1978 to 1979 and from 1983 to 1988, for Plastic from 1979 to 1988, for Crosson from 1980 to 1988, and Jerry from 1978 to 1980. Data are expressed on the basis of hydrological years, i.e., 1978 includes June 1, 1978, to May 31, 1979.

A second set of study lakes (B lake data set) included 10 lakes (Table 1) for which Fe concentrations as well as morphometric data were measured and hydrologic data estimated as part of the study. These lakes were used to test several hypotheses described later.

A third set (C lake data set) consisted of lake data gleaned from the published literature (Table 2). This set included five lakes in the Sudbury area, Ontario (Yan and Miller 1984; Dillon et al. 1988), that experience substantial acid and heavy metal pollution from local smelters. All of these lakes except Clearwater had undergone various liming operations to chemically neutralize the water with $CaCO_3$ and/or $Ca(OH)_2$. Data for eight additional lakes included two lakes in the Experimental Lakes Area (ELA), northern Ontario, two lakes from the Adirondacks of New York State, one additional lake from the Canadian Shield, Ontario, and two European lakes. Data sets A and B offer the most reliable information because the data were usually collected over a long period (typically 10 or more years) and similar methods were used throughout. Therefore, emphasis is given to results based

TABLE 2. Location and morphometrical characteristics of lakes from the literature (C Lakes). All of the Sudbury lakes except Clearwater had been treated with $CaCO_3$ and/or $Ca(OH)_2$ in recent years. na, data not available. Won., Wononscopomuc, deep and shallow basins. Key to references (Ref.): 1, Cross and Rigler (1983); 2, Cook (1984); 3, Davison et al. (1980); 4, Löfgren (1987); 5, Nürnberg (1984b, 1984c); 6, Lee (1962); Stauffer (1986); 7, Dean and Greeson (1979); 8, Effler et al. (1981); 9, Schindler et al. (1976); 10, Nürnberg et al. (1987); 11, Dillon et al. (1988) and Yan and Dillon (1984).

Lake	Latitude	Longitude	A_0 (ha)	$\bar{z}$ (m)	τ_w (yr)	q_s (m/yr)	Ref.
C lakes							
Bob	44°55′N	78°47′W	228	18.0	2.99	6.02	1
Lake 227 (ELA)	49°41′N	93°40′W	5	4.4	2.15	2.05	2
Esthwaite	54°21′N	3°00′W	100	6.4	0.25	25.60	3
Finjasjoen[a]	56°08′N	13°42′W	1110	3.8	0.33	11.51	4
Jack's	44°41′N	78°02′W	na	8.8	8.00	1.10	5
Magog	45°18′N	72°03′W	1044	9.8	0.06	163.33	5
Memphramagog	45°05′N	72°15′W	280	10.0	na	na	5
Mendota	45°30′N	89°25′W	3910	12.4	5.50	2.26	6
Oneida[a]	43°15′N	76°00′W	20700	6.8	0.67	10.15	7
Onondaga	43°00′N	76°10′W	1170	12.0	0.27	44.44	8
Rawson (ELA)	49°40′N	93°45′W	54	10.5	6.35	1.65	9
St. George, East	43°57′N	79°25′W	5	5.5	2.30	2.40	5
St. George, West	43°57′N	79°25′W	4	5.2	2.16	2.40	5
Waramaug	41°30′N	73°22′W	272	7.0	0.83	8.43	10
Won., deep	41°45′N	73°20′W	70	12.5	4.00	3.12	10
Won., shallow	41°45′N	73°20′W	24	8.5	4.00	2.12	10
Sudbury lakes							
Clearwater	46°22′N	81°03′W	76.5	8.3	4.40	1.89	11
Hannah	46°27′N	81°02′W	27.3	4.0	3.00	1.33	11
Lohi	46°23′N	81°02′W	40.5	6.2	1.30	4.77	11
Middle	46°26′N	81°02′W	28.2	6.2	1.80	3.44	11
Nelson	46°44′N	81°05′W	309.0	11.6	9.30	1.25	11

[a]Polimictic, not stratified.

on these data sets. Data set C is used to test several of the hypotheses, although it should be considered that any differences in the results may be attributable to the different methods as well as to different lake characteristics and geography.

Mass Balance Calculations

The discharges of all major inflows (two to six per lake) and the outflows of each A lake were monitored for the duration of the study; water level was continuously recorded in each inflow and converted to discharge using a site-specific stage–discharge relationship derived from 300–500 instantaneous measurements per site. Methods are described in detail in Scheider et al. (1983) and Locke and Scott (1984). The lake water budgets, including precipitation directly to the surface of the lakes and lake evaporation estimated from meteorological data, were balanced to within 10% in 80 of 81 lake-years.

All inflow and outflow samples were retrieved at or below the notch of the wire or within the flume of the streams. Sampling frequency varied from biweekly or monthly during base flow conditions in the winter to two or three times per week during spring thaw and weekly during the summer.

Because there were no significant relationships between stream discharge and stream metal concentration, mass input (milligrams per year) was calculated by multiplying the concentration of the metal in the stream measured during the midpoint of a time period by the total discharge over that time interval. Flux of Fe into each lake from the portion of the lake's catchment that could not be gauged hydrologically (15–70%) was estimated by prorating the measured input from that lake's tributaries on an areal basis. The direct input of Fe to each lake in precipitation falling on the lake's surface was measured (Jeffries 1984; Dillon et al. 1988), combined with the input from the catchment, and divided by the lake surface area (A_0) to yield external Fe load (L_{ext}, milligrams per square metre per year). The mass leaving the lake via the outflow divided by A_0 is called L_{out} (milligrams per square metre per year).

Internal load (L_{int}), i.e., the load from internal Fe sources (presumably the reduced sediment surfaces), was determined independently. The changes in the Fe mass of the whole water column during the stratification period between June 1 and October 31 in all lakes but shallow Dickie (June 1 to September 30) (ΔFe_{Lake}) were corrected for differences between the external input and output via outflow during this period (ΔFe_{in-out}). Division by A_0 then yields L_{int} (milligrams per square metre per summer)

$$(1)\quad L_{int} = (\Delta Fe_{Lake} - \Delta Fe_{in-out})/A_0 .$$

Because winter Fe release appeared to be negligible in all but a basin of Red Chalk Lake (P. Dillion, unpublished data), we consider L_{int} to represent the annual internal input. Internal load determined this way represents a net internal input. Any cycling and redistribution after precipitation in the stratified hypolimnion was not considered. Furthermore, any oxic release during the winter or during destratified periods has been considered to be minor compared with the Fe release during summer. The total load, L_{tot} (milligrams per square metre per year), is calculated as the sum of L_{ext} and L_{int}.

Fe retentions were computed from the mass balance data in two ways:

$$(2)\quad R_{ext} = (L_{ext} - L_{out})/L_{ext}$$

and

$$(3)\quad R_{tot} = (L_{tot} - L_{out})/L_{tot}\,.$$

R_{ext} is the retention commonly estimated in lakes for various substances. It does not consider L_{int} and represents net retention including downward fluxes and fluxes upwards from the sediment. R_{tot}, on the other hand, includes L_{int}. It consists mainly of downward fluxes and on an annual basis therefore resembles more closely gross retention.

A simple one-box mass balance model was applied to the data, similar to that of Dillon and Rigler (1975):

$$(4)\quad TFe_{pred} = L_{ext}\,(1 - R_{ext})/q_s$$

or

$$(5)\quad TFe_{pred} = L_{tot}\,(1 - R_{tot})/q_s$$

where q_s represents annual water load (metres per year). Both expressions are mathematically identical and TFe can be computed from the mass balance data by substituting Eq. 2 or Eq. 3 into Eq. 4 or Eq. 5, respectively, to render Eq. 6:

$$(6)\quad TFe_{pred} = L_{out}/q_s.$$

Equation 6 also states that TFe_{pred} is equal to the volume-weighted average Fe concentration in the outflow, a variable that can more easily be assessed than the other budget components.

Lake Concentrations of Fe and Sediment Samples

The concentration of Fe in each lake was measured 8–30 times per year. During the period of thermal stratification, volume-weighted samples were collected from the epi-, meta-, and hypolimnion by combining aliquots taken every 2 m. The resulting measured concentrations in the three strata were then combined to give volume-weighted mean whole-lake concentrations. Samples were collected throughout the entire water column when the lakes were homothermal. From these data, annual average whole-lake Fe concentrations (TFe) were computed as the grand mean. TFe is probably the most informative and exact measure of Fe quantity in a lake, since it is assembled from numerous measurements in time and space. However, Fe concentrations during the two mixed periods might equally well represent the Fe quantity in a lake and are easier to determine than TFe. Therefore, spring and fall Fe concentrations were each calculated separately as averages during the periods when the lakes were destratified.

Data on sediment LOI originated in cores taken during summer stratifications between 1979 and 1986 from the profundal sediment of each lake (Smith 1983; Nürnberg 1988 and unpublished data). Typically, averages of at least two cores were taken. The LOI values reported here represent average values of the 0–10 cm surficial sediments analyzed in separate 1- or 5-cm slices.

Analytical

Fe concentrations in stream, lake, and precipitation samples, DOC concentrations, and water colour of the lakes were measured following methods outlined in detail by the Ontario Ministry of the Environment (1983). From 1980 to 1985, total Fe was colorimetrically determined after acid reduction by formation of the ferrous-2,4,6-tri-(2′pyridyl-)-1,3,5-triazine complex and is accurate to 0.02 mg/L. From 1985, acid digestion followed by atomic adsorption spectroscopy was employed with a precision of 0.005 mg/L. The two methods yielded results that were identical. (Slope of the linear regression of one method on the other was not significantly different from 1.0, and the intercept was not significantly different from 0.0.) LOI was determined with standard methods by igniting aliquots of the sediment samples at 500°C to constant weight.

Results and Discussion

Annual Average Fe Concentration (TFe)

TFe in the combined lake set (A, B, and C data sets) ranged from 20 to 550 µg/L (Table 3). The A lakes had average TFe concentrations between 70 and 300 µg/L, while concentrations in two B lakes and one C lake (polymictic Finjasjoen) exceeded 500 µg/L. TFe values for up to 11 yr each are available for the eight A lakes and add up to 79 individual lake-years. The TFe values give an indication of the annual variation of the data. The year to year variation can be substantial (the coefficient of variance (CV) is mostly between 0.20 and 0.35) but is still lower than the lake to lake variation of TFe (CV of 0.60). Most of this Fe must be complexed with organic acids as long as it remains in oxic waters, since theoretically, dissolved uncomplexed Fe concentrations at the prevailing freshwater conditions (pH > 4.8 and redox potential E_H > 200 mV) cannot exceed 10 µg/L (Mill 1980).

Fe concentrations are typically below 30 µg/L in lakes that develop hydrogen sulfide (H_2S) in their anoxic hypolimnia. In these lakes, H_2S precipitates Fe as ferrous sulfide (FeS) to form a black layer above or in the sediment. Typically, these lakes are well buffered and pH is at neutral or above. The Fe cycle of these H_2S-containing lakes (Table 3) is different from those of the lakes that are oversaturated with Fe with respect to H_2S. Retention of Fe ought to be close to 1.0 due to FeS formation and biological uptake (Murphy and Yesaki 1983), and L_{int} should be close to zero, despite anoxia. There is only one Fe budget available for a sulfide-rich lake, Lake Mendota, where L_{int} was assumed to be zero and R_{ext} was estimated as 0.99 (Stauffer 1986). Analytical limits render Fe budgets in such an Fe-poor lake difficult or impossible to establish. For these reasons, this study concentrates on lakes with TFe above 30 µg/ L with the exception of Nelson Lake in the Sudbury region. Its low TFe concentration was artificially created by liming that reduced the TFe concentration from 61 to 20 µg/L and is not due to FeS precipitation (Yan and Dillon 1984; Table 3).

Average annual TFe concentrations are higly correlated with spring turnover concentrations ($r^2 = 0.91$, $n = 16$, F-ratio = 134); paired t-test and Wilcoxon's nonparametric test both show no significant difference between the two (Fig. 1). On the other hand, fall turnover concentrations are significantly higher than TFe concentrations and their correlation with TFe is not as good ($r^2 = 0.69$, $n = 26$, F-ratio = 54). Therefore, spring Fe concentrations can be substituted for annual TFe if annual average concentrations are not available, but fall concentrations cannot.

TABLE 3. Average annual whole-lake Fe concentration (TFe), pH, organic carbon (DOC and colour), and LOI of the surface sediments. na, data not available. Data correspond to the years for which budgets are available, whenever possible.

Lake	TFe (µg/L)	pH	DOC (mg/L)	Colour (Hazen)	LOI (%)
A and B lakes					
Blue Chalk	70	6.61	1.79	5	33
Chub	273	5.62	4.74	33	50
Crosson	263	5.58	4.06	23	41
Dickie	276	5.80	5.10	35	37
Harp	106	6.28	3.86	20	34
Jerry	213	6.09	4.76	na	38
Plastic	106	5.73	2.24	5	42
Red Chalk	120	6.32	2.49	9	41
Basshaunt	80	6.68	4.16	24	na
Bigwind	100	6.33	3.17	9	na
Buck	120	6.49	2.82	5	41
Glen[a]	30	7.70	3.78	8	na
Gravenhurst Bay	162	7.00	3.60	na	28
Gullfeather	550	5.90	5.14	51	na
Little Clear	520	6.58	2.66	10	45
Paint-10	200	5.30	4.30	20	61
Solitaire	40	6.58	2.11	5	37
Walker	70	6.55	3.69	10	15
C lakes					
Bob	na	6.67	na	na	30
Dart's	na	5.10	na	na	na
Lake 227 (ELA)	na	6.20	na	18	48
Esthwaite	na	na	na	na	24
Finjasjoen	550	8.90	na	na	na
Jack's[a]	20	7.00	na	11	na
Magog	140	7.50	na	na	na
Memphramagog	35	7.50	na	na	na
Mendota[a]	na	8.50[b]	Low	Low	na
Oneida	36	8.10	na	na	na
Onondaga	na	na	na	na	na
Rawson	na	6.20	na	na	31
St. George, East[a]	20	7.40	6.50	24	32
St. George, West[a]	20	7.40	7.60	25	28
Waramaug	na	6.40	3.70	na	23
Wononscopomuc[a]	20	7.00	3.00	na	31
Won., shallow[a]	20	6.90	2.80	na	32
Sudbury lakes					
Clearwater	88	4.40	0.50	na	20
Hannah	169[c]	6.70	0.50	na	20
Lohi	72[c]	4.75	0.50	na	24
Middle	106[c]	6.70	0.50	na	28
Nelson	20[c]	6.70	0.50	na	na
Mean	144	6.5	3.24	17.6	34
Range	20–550	4.4–8.9	0.5–7.6	4.6–51.4	15–61
SE	26.5	0.14	0.34	2.76	2.0

[a]Hydrogen sulfide lakes.
[b]pH value for Mendota is taken at fall turnover.
[c]Preliming concentrations: Hannah, 104; Lohi, >90; Middle, na; Nelson, 61.

Organic Acid Indicators

Since we presume that the Fe cycle is substantially influenced by humic and fulvic acids, we report all organic acid indicators that were available to us, i.e., average DOC concentration and colour intensity of the lake water as direct measures and LOI of the sediment as a more indirect measure (Table 3). We assumed that in the A and B lakes, LOI represents mainly settled humic and fulvic acids and only a minor part may consist of biological residue. To test this assumption, we correlated the organic acid indicators among themselves. Of DOC, colour, and LOI, DOC and colour are significantly correlated with each other ($r^2 = 0.50$, $n = 18$, $p < 0.001$, F-ratio = 16). This regression is even more significant when lakes with TFe below 30 µg/L are excluded ($r^2 = 0.82, n = 15, p < 0.0001, F$- ratio = 59). This good correlation means that in this subset of lakes, DOC and colour are interchangeable and indeed indicate humic and fulvic acids. However, sediment LOI is correlated with neither DOC nor colour. There may be two reasons for the lack of a correlation of

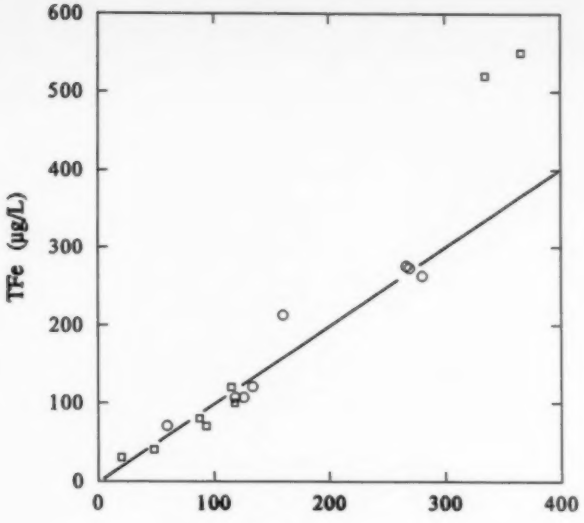


FIG. 1. Average annual and average spring Fe concentration of A (circles) and B (squares) lakes. The 1:1 line is shown.

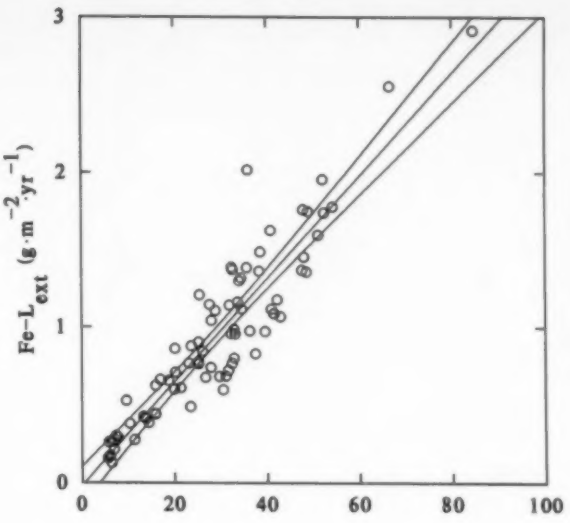


FIG. 2. External loads of Fe and DOC for 76 lake-years of the eight A lakes. The regression line and 95% confidence limits are shown for Fe-L_{ext} = –0.017 (0.05) + 0.033 (0.002) DOC-L_{ext} ($r^2 = 0.85$, $n = 76$, $p < 0.0001$) (SE in parentheses).

LOI. It could be due to the different origin of the samples, sediment versus lake water, or it could mean LOI includes biomass and detritus as well and is less useful in indicating humic and fulvic acids even in these oligotrophic lakes.

There is a strong correlation between TFe and colour for the A lakes: TFe = 53 + 6.5 colour ($n = 7$, $r^2 = 0.80$, $p < 0.007$). When the remaining data or all data together are used, significant multiple regressions including colour and the annual water load, q_s, were obtained ($r^2 = 0.70$, $n = 11$, $p < 0.008$ or $r^2 = 0.67$, $n = 18$, $p < 0.0001$). The addition of water load to the regression corrects for dilution effects in lakes with different hydrology. These good relationships with colour can be explained by the tendency of humic or fulvic acids to associate with iron oxyhydroxides. The organic acids keep the brown-red iron hydroxide particles in suspension that would otherwise (in clear waters) precipitate and settle to the bottom. The presence of colour therefore indicates that both Fe and organic acids are present.

Like colour, DOC ($r^2 = 0.49$, $n = 26$, $p < 0.0001$) and even LOI ($r^2 = 0.68$, $n = 20$, $p < 0.0001$) are positively correlated with TFe in multiple regressions with q_s as an additional variable, supporting our view that organic acids affect the Fe cycle in lakes.

Mass Balances

Mass balance components were available for A and some C lakes only. External Fe loads were highly variable (Table 4). Most of the external Fe input stemmed from terrestrial sources for all lakes but the Sudbury lakes. Here, up to a third of L_{ext} (12–33%) originated from smelters and entered the lake via precipitation directly on the surface. In the A lakes, only 8% on average (2–20%) was atmospheric load. Much of the Fe entering the A lakes originated in the surrounding wetland areas and swamps and is probably associated with fulvic and humic acids (LaZerte 1991). This view is strongly supported for the A lakes for which DOC budgets are available (P. Dillon, unpublished data). Here the correlation between annual external loads of DOC and Fe is highly significant ($r^2 = 0.85$, $n = 76$, $p < 0.0001$; Fig. 2).

Internal Fe load represents Fe that originated in the sediment or on the sediment surface and that was released or dissolved when the redox potential (E_H) dropped to below 200 mV. Of the A and B lakes, all but Harp Lake exhibited significant hypolimnetic oxygen depletion during the summer. The average summer anoxic factors, which measure the extent of anoxia in space and time and represent the number of days that an area equivalent to the whole-lake surface area becomes anoxic (Nürnberg 1987), ranged between 11 and 27 d per summer for these lakes but only 3.5 d for Harp. Except in Harp Lake, the E_H values measured 1 m above the bottom in the late summer at a deep spot were below 100 mV. The lowest (24 mV) was recorded in Blue Chalk Lake. L_{int} (Table 4) appears to be an important factor in the Fe budgets; it is highly variable, independent of L_{ext}, and can be up to four times larger than L_{ext} (Blue Chalk).

The Fe loss via outflow (L_{out}, Table 4) used to calculate Fe retention is typically much lower than I_{ext} and never larger when the average L_{out} of several years is used; in other words, at least some Fe on average is lost to the sediments in all lakes, and R_{ext} is positive. If individual years are considered, more Fe flows out of Chub, Crosson, and Dickie lakes for 2 yr than comes in, and R_{ext} becomes negative in these cases. This phenomenon can be explained by high sediment release of Fe and high content of organic matter that keeps released Fe in suspension. DOC is indeed relatively high in these lakes (4–5 mg/L, Table 3). In the same years, R_{tot} still remains positive and in fact never becomes negative but ranges from 0.19 to 0.98 with an average of 0.72. Annual values of R_{ext}, on the other hand, range from –0.19 to 0.83, with an average of 0.43.

For several substances, especially for total phosphorus in lakes without internal phosphorus load (i.e., where R_{ext} equals R_{tot} as in oxic lakes, Nürnberg 1984a), and for the three nitrogen

TABLE 4. Fe budget data. Averages of annual observations for specified years are presented (years). All loads in $mg \cdot m^{-2} \cdot yr^{-1}$, L_{out}/q_s, model of Eq. 5; na, data not available.

Lake	L_{ext}	L_{int}	L_{out}	L_{out}/q_s	L_{tot}	R_{ext}	R_{tot}	Years
A lakes								
Blue Chalk	269	1043	81	50	1311	0.68	0.94	1978–88
Chub	1109	1597	881	194	2706	0.18	0.65	1978–88
Crosson	1322	1635	1198	210	2963	0.08	0.51	1980–88
Dickie	1021	1182	621	207	2204	0.34	0.71	1978–88
Harp	1266	0	456	103	1266	0.64	0.64	1978–88
Jerry	2735	295	1407	142	3030	0.48	0.53	1978–79
Plastic	501	883	239	110	1384	0.52	0.83	1979–88
Red Chalk	862	891	464	81	1753	0.45	0.74	1978–79, 1983–88
C lakes								
Clearwater	1856	0	363	192	1856	0.80	0.80	1977
Hannah	894	0	242	182	894	0.73	0.73	1977
Lohi	1620	na	770	161	na	0.52	na	1977
Middle	1525	na	567	165	na	0.63	na	1977
Nelson	485	0	74	59	485	0.85	0.85	1977
Bob	4209	0	985	164	4209	0.77	0.77	1975
Lake 227 (ELA)	1431	8932	na	na	10363	na	na	1979
Esthwaite	20000	2700	6400	250	22700	0.68	0.72	1978
Finjasjoen	6500	8514	5455	474	15014	0.16	0.64	1982–83
Mendota	12800	0	130	20	12800	0.99	0.99	1977
Oneida	974	0	386	38	974	0.60	0.60	1979
Onondaga	45236	na	na	na	na	na	na	1969, 1975
Rawson	647	0	94	57	647	0.85	0.85	1972–73
Mean	5108	1537	1095	150	4809	0.58	0.73	
Range	239–45235	0–8932	46–6400	20–474	485–22700	0.08–0.85	0.51–0.94	
SE	2262.1	642.7	401.9	23.7	1466.1	0.057	0.032	

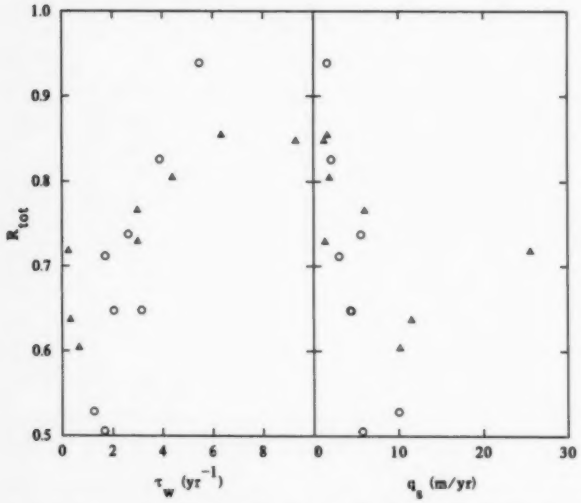


FIG. 3. R_{tot} versus τ_W and q_s. The A lakes (circles) and literature data (C lakes, triangles) are shown.

fractions of nitrate, ammonium, and total nitrogen (Dillon and Molot 1990), strong correlations with morphometry and hydrology of the lake are evident. Therefore, we tested the hypothesis that Fe retention is correlated with morphometric and hydrological lake characteristics as well. Since R_{ext} is obscured by L_{int}, because Fe released from sediments is not incorporated in its computation, we did not expect any support of that hypothesis and indeed we did not find any relationship between annual water load, q_s, or annual water detention time, τ_w and R_{ext}. Instead, we found a negative correlation with L_{int} for A lakes ($r^2 = -0.58, n = 8, p < 0.003$). The larger is L_{int}, the more Fe leaves the lake, and the lower is the apparent or net retention (R_{ext}). On the other hand, R_{tot}, which resembles gross retention, depends on morphometry and hydrology and is indeed correlated with τ_w and q_s (Fig. 3). These results demonstrate the importance of considering Fe release from sediment surfaces. If only the commonly used R_{ext} had been applied, the unconsidered influence of L_{int} on retention would have obscured the hydrological and morphological dependence of the retention.

Settling Velocity

The observed relationship between R_{tot} and q_s has the additional benefit of providing the means for evaluating the settling velocity of Fe, since

$$(7) \quad 1/R_{tot} = 1 + 1/S \times q_s$$

where S is the average net mass transfer coefficient (metres per year) (Kelly et al. 1987; Dillon and Molot 1990). Assuming that all unconsidered fluxes, such as Fe release, during the unstratified or winter season are minor, this coefficient approximates the settling velocity. Using all the available data except one outlier (Esthwaite), the equation is $1/R_{tot} = 1.18\ (0.087) + 0.054\ (0.015)\ q_s$, ($r^2 = 0.49, n = 15, p < 0.003$) (SE in

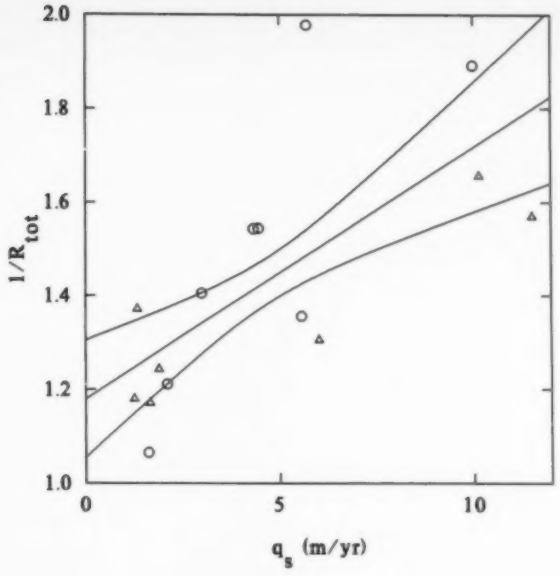


FIG. 4. Reciprocal of R_{tot} versus q_s. The A lakes (circles) and literature data (C lakes, triangles) are shown. Lake Esthwaite is excluded from the regression and plot as an significant outlier (data pair 25.6/1.39). The regression line and 95% confidence limits are shown.

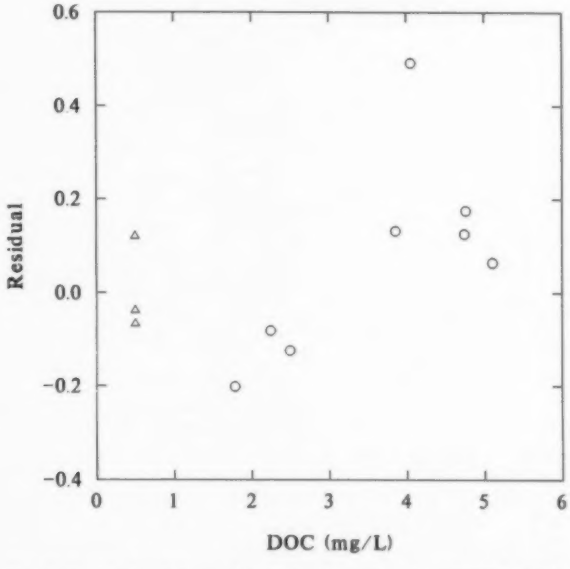


FIG. 5. Residuals of the model of Fig. 4 as a function of DOC. A lakes (circles) and literature data without Esthwaite (triangles) are shown.

parentheses) (Fig. 4). The constant is not significantly different from 1, and the reciprocal of the slope gives an average transfer coefficient of 18.5 m/yr; if forced through a constant of 1, settling velocity is 12.7 m/yr. For the A lakes, S is 10.5 ($1/R_{tot}$ = 1.06 (0.15) + 0.095 (0.029) q_s, $r^2 = 0.64$, $n = 8$, $p < 0.017$). For the C data set only, including three Sudbury lakes, but without Esthwaite, S is estimated as 26.3; however, the constant is

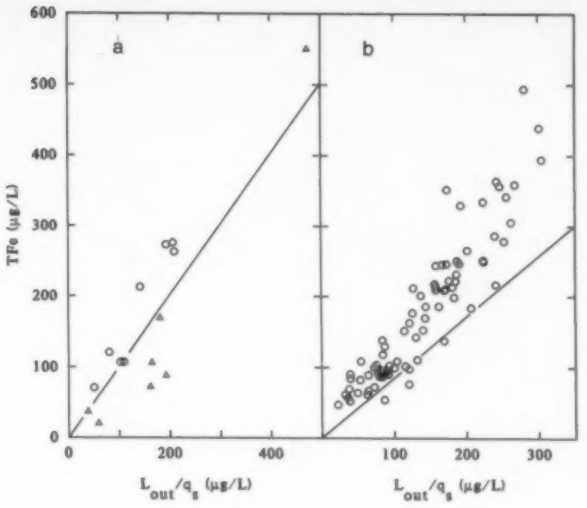


FIG. 6. TFe versus average outflow concentration (L_{out}/q_s) (a) for averages of all the lakes and (b) for all individual lake-years of the A lakes. Lines of perfect fit for A lakes (circles) and literature data (triangles) are shown.

slightly higher than 1 ($1/R_{tot}$ = 1.17 (0.006) + 0.038 (0.009) q_s, $r^2 = 0.78$, $n = 7$, $p < 0.009$).

The Fe settling velocity is similar to those determined for phosphorus, which has been reported as 10 (Vollenweider 1968), 13.2 (Dillon and Kirchner 1975), and 16 m/yr (Chapra 1975) or even higher (Nürnberg 1984a). We believe that in low-DOC lakes, the settling velocity is higher for Fe than for phosphorus because precipitation of oxidized Fe occurs more rapidly than the biological sedimentation of phosphorus; however, in lakes coloured with dissolved organic matter, Fe may stay in solution even after aeration. Experimental support is given by Sigg (1987) who determined that summer sedimentation of Fe into sediment traps in the clear hardwater Lakes Zürich and Constance was two and four times respectively, larger, than that of phosphorus on molar basis. Particle stability as a measure of how long a particle stays intact is an order of magnitude lower in these clear lakes (DOC < 1 mg/L) than in coloured Swiss lakes (DOC) 3–5 mg/L, O'Melia 1987), hence favouring sedimentation in clear lakes.

The dependence of retention on DOC or colour in our study is supported by residual analysis of the model $1/R_{tot} = f(q_s)$. Without the literature data, residuals increase with DOC (Fig. 5) and colour. However, the residuals are not affected by internal load, suggesting that gross retention, i.e., sedimentation, is similar for both external and internal Fe load. The lower sedimentation rate in the A lakes is probably the result of humic and fulvic acids. Lower sedimentation was also observed by Urban et al. (1990) in brown water of Nova Scotia lakes with pH values comparable with those of the A lakes (pH > 5).

Iron Mass Balance Model

A simple one-box mass balance model (Eq. 4, 5, or 6) was used to predict Fe concentrations in all the lakes for which the information was available, i.e., 15 A and C lakes. The computed Fe

concentrations (L_{out}/q_s, Table 4) are compared with observed annual Fe concentrations for lake averages (TFe, Table 3; $r^2 = 0.82$, $n = 15$, $p < 0.0001$, Fig. 6a) and individual lake years ($n = 79$, $p < 0.0001$, Fig. 6b). The model underestimates average TFe in all lakes with higher DOC values by 21% on average (i.e., all but Sudbury Lakes, paired t-test, $n = 10$, $p < 0.004$) and in all individual years of the A lakes by 25% (paired t-test, $n = 79$, $p < 0.0001$). This underestimate results partly because the volume-weighted average Fe concentration in the lake is elevated by high Fe concentration in the anoxic hypolimnion, which does not appear in the outflow except in the fall during overturn.

In the lakes with low DOC concentrations, like the Sudbury lakes, the model overestimates by 67% ($n = 5$, $p < 0.02$) on average. The different model results may reflect differences in the source of Fe (anthropogenic and airborne), different chemistry (very low DOC and pH), and past management. For example, the observed TFe concentration had been artificially reduced due to chemical neutralization with $CaCO_3$ and/or $Ca(OH)_2$ in at least two of the four treated lakes (Table 3). Another possible explanation is a low settling velocity caused by the photochemical reduction of iron hydroxides to more soluble ferrous iron. This has been observed in the laboratory (Waite and Morel 1984), in water from an acidic bog lake in Ohio (Francko and Heath 1982), and in a clear, acid mountain lake in Switzerland (Sulzberger et al. 1990).

It can be concluded that the simple mass balance model gives proportionate but not quantitative estimates of the average annual Fe concentration. In lakes with natural Fe sources, the model tends to underestimate Fe concentration, while in acid lakes with high airborne anthropogenic load the model tends to overestimate it. Since spring Fe concentrations available for the A lakes are not significantly different from TFe data, they are correlated with the model estimates as well ($r^2 = 0.95$, $n = 8$, $p < 0.0001$), with an average of 22% lower estimates.

No matter what the reason is for the deviation of the predicted from observed Fe concentrations, the mass balance model can be turned into a more general model that quantitatively predicts TFe. We achieved this by inclusion of an organic matter term because the residuals of the mass balance model are significantly correlated with DOC concentration and LOI of the sediment for the combined data set. The addition of DOC to the mass balance model improves r^2 from 0.49 ($n = 13$) to 0.90 and the F-ratio from 10 to 44 in TFe = –43.9 (24) + 0.81 (0.16) L_{out}/q_s + 30.0 (4.8) DOC ($p < 0.0001$) (SE in parentheses). The addition of LOI improves r^2 from 0.39 ($n = 12$) to 0.80 and the F-ratio from 6 to 18 in TFe = –190.7 (60) + 1.07 (0.23) L_{out}/q_s + 5.47 (1.3) LOI ($p < 0.001$). Paired t-tests indicate the lack of any significant differences between predicted and observed Fe concentrations. These models (Fig. 7) now apply to coloured lakes with natural tributary input as well as to clear, acid-stressed lakes with anthropogenic airborne Fe sources. Since the term L_{out}/q_s is equivalent to average Fe outflow concentration, the models should be useful in determining average Fe concentrations in lakes in a relatively simple and cost-effective manner.

Conclusion

This analysis is the first step in evaluating important parameters of the Fe cycle in lakes, such as the average settling velocity of Fe and its dependence on organic acids, and in comparing these parameters between lakes. For example, the analysis of both measures of retention, the commonly used R_{ext} and the introduced R_{tot}, shows how important it is to consider internal load. Only R_{tot} demonstrates the expected dependency on hydrology and morphometry.

Many of the concepts and approaches have been borrowed from the studies of phosphorus cycling in lakes (e.g., Nürnberg 1984a, 1991). It appears natural that similar phenomena occur with substances that, like phosphorus and Fe, experience a redox-controlled sediment release. Our empirical equations should make it practical for other workers to compare and apply the equations to their own systems. For example, the management of drinking water impoundments may be facilitated by the application of our results.

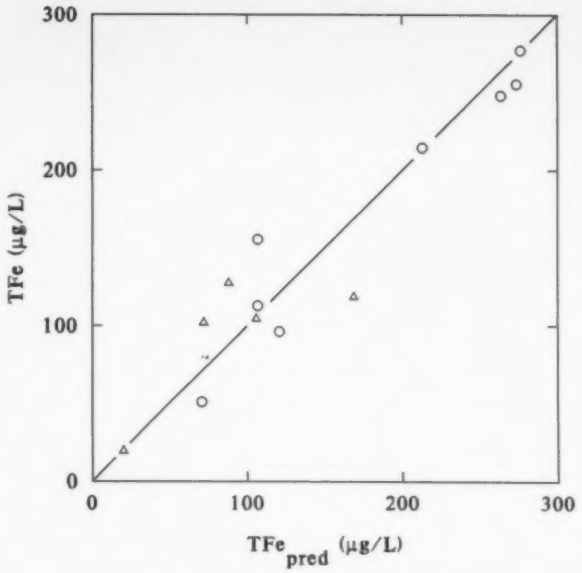


FIG. 7. TFe concentrations versus predictions from the model $TFe_{pred} = -43.9 + 0.81\ L_{out}/q_s + 30.0$ DOC ($r^2 = 0.90$, $n = 13$, $p < 0.0001$). The line of perfect prediction for A lakes (circles) and literature data (triangles) is shown.

Acknowledgements

This study was supported by an Ontario Ministry of the Environment grant to Don McQueen, York University, and the first author. We thank R. Girard, R. Reid, and L. Scott for invaluable assistance in conducting the field work described herein. R. Stauffer provided valuable insight into Lake Mendota data. Richard Carignan and Robert Peters contributed excellent ideas to the final stages of this manuscript.

References

BACCHINI, P., AND T. JOLLER. 1981. Transport processes of copper and zinc in a highly eutrophic and meromictic lake. Swiss J. Hydrol. 43: 176–199.

CHAPRA, S.C. 1975. Comment on "An empirical method of estimating retention of phosphorus in lakes" by W.B. Kirchner and P.J. Dillon. Water Resour. Res. 11: 1033–1034.

COOK, R.B. 1984. Distribution of ferrous iron and sulfide in an anoxic hypolimnion. Can. J. Fish. Aquat. Sci. 41: 286–293.

COOK, R.B., C.A. KELLY, D.W. SCHINDLER, AND M.A. TURNER. 1986. Mechanisms of hydrogen ion neutralization in an experimentally acidified lake. Limnol. Oceanogr. 31: 134–148.

CROSS, P.M., AND F.H. RIGLER. 1983. Phosphorus and iron retention in sediments measured by mass budget calculations and directly. Can. J. Fish. Aquat. Sci. 40: 1589–1597.

DAVIS, J.A. 1982. Adsorption of natural dissolved organic matter at the oxide/water interface. Geochim. Cosmochim. Acta 46: 2381–2393.

DAVISON, W., S.I. HEANEY, J.F. TALLING, AND E. RIGG. 1980. Seasonal transformations and movements of iron in a productive English lake with deep-water anoxia. Swiss J. Hydrol. 42: 196–224.

DEAN, W.E., AND P.E. GREESON. 1979. Influences of algae on the formation of freshwater ferromanganese nodules, Oneida Lake, New York. Arch. Hydrobiol. 86: 181–192.

DILLON, P.J., H.E. EVANS, AND P.J. SCHOLER. 1988. The effect of acidification on metal budgets of lakes and catchments. Biogeochemistry 5: 201–220.

DILLON, P.J., AND W.B. KIRCHNER. 1975. Reply. Water Resour. Res. 11: 1035–1036.

DILLON, P.J., AND L.A. MOLOT. 1990. The role of ammonium and nitrate retention in the acidification of lakes and forested catchments. Biogeochemistry 11: 23–43.

DILLON, P.J., AND F.H. RIGLER. 1975. A simple method for predicting the capacity of a lake for development based on lake trophic status. J. Fish. Res. Board Can. 32: 1519–1531.

EFFLER, S.W., S.D. FIELD, M.A. MEYER, AND P. SIZE. 1981. Response of Onondaga Lake to restoration efforts. J. Environ. Eng. ASCE 107: 191–210.

FRANCKO, D.A., AND R.T. HEATH. 1982. UV-sensitive complex phosphorus: association with dissolved humic material and iron in a bog lake. Limnol. Oceanogr. 27: 564–569.

GUNKEL, G., AND A. SZTRAKA. 1986. Investigations about the fate of heavy metals in lakes. II. The role of iron and manganese remobilization to the hypolimnic enrichment of heavy metals. Arch. Hydrobiol. 106: 91–117.

JEFFRIES, D.S. 1984. Atmospheric deposition of pollutants in the Sudbury area, p. 118–155. *In* J. Nriagu [ed.] Environmental impacts of smelters. John Wiley & Sons, Inc., New York, N.Y.

KELLY, C.A., J.W.M. RUDD, R.U. HESSLEIN, D.W. SCHINDLER, P.J. DILLON, C.T. DRISCOLL, S.A. GHERINI, AND R.E. HECKY. 1987. Prediction of biological acid neutralization in lakes. Biogeochemistry 3: 129–140.

KIRCHNER, W.B., AND P.J. DILLON. 1975. An empirical method of estimating the retention of phosphorus in lakes. Water Resour. Res. 11: 182–183.

KHOE, G.H., AND T.D. WAITE. 1989. Manganese and iron related problems in Australian water supplies. Environ. Technol. Lett. 10: 479–490.

LAZERTE, B. 1991. Metal transport and retention: the role of dissolved organic carbon. Finn. Humus News 3: 71–82.

LEE, G.F. 1962. Studies on the iron, manganese, sulfate and silica balances and distributions for Lake Mendota, Madison, Wisconsin. Wis. Acad. Sci. Arts Lett. 51: 141–155.

LIANG, L., AND J.J. MORGAN. 1990. Chemical aspects of iron oxide coagulation in water: laboratory studies and implications for natural systems. Aquat. Sci. 52: 32–55.

LOCKE, B.A., AND L.D. SCOTT. 1984. Studies of lakes and watersheds in Muskoka–Haliburton, Ontario: methodology (1976–1985). Ont. Minist. Environ. Data Rep. DR 83/1. 1983.

LÖFGREN, S. 1987. Aerobic sediment iron and phosphorus release in Lake Finjasjön, a shallow, eutrophic Swedish Lake. Ph.D. thesis, Uppsala, Sweden.

MILL, A.J.B. 1980. Colloidal and macromolecular forms of iron in natural waters. 1. A review. Environ. Technol. Lett. 1: 97–108.

MOREL, F.M.M., AND P.M. GESCHWEND. 1987. The role of colloids in the partitioning of solutes in natural waters, p. 405–422. *In* W. Stumm [ed.] Aquatic surface chemistry: chemical processes at the particle–water interface. John Wiley & Sons, Inc., New York, N.Y.

MURPHY, T.P., AND I. YESAKI. 1983. Biogenic regulation of iron availability in a eutrophic hardwater lake. Sci. Total Environ. 28: 37–50.

NÜRNBERG, G.K. 1984a. The prediction of internal phosphorus load in lakes with anoxic hypolimnia. Limnol. Oceanogr. 29: 11–124.

NÜRNBERG, G.K. 1984b. Iron and hydrogen sulfide interference in the analysis of SRP in anoxic waters. Water Res. 20: 369–377.

NÜRNBERG, G.K. 1984c. The availability of phosphorus from anoxic hypolimnia to epilimnetic plankton. Ph.D. thesis, McGill University, Montreal, Que. 240 p.

NÜRNBERG, G.K. 1987. A comparison of internal phosphorus loads in lakes with anoxic hypolimnia: laboratory incubation versus in situ hypolimnetic phosphorus accumulation. Limnol. Oceanogr. 32: 1160–1164.

NÜRNBERG, G.K. 1988. The prediction of phosphorus release rates from total and reductant-soluble phosphorus in anoxic lake sediments. Can. J. Fish. Aquat. Sci. 45: 453–462.

NÜRNBERG, G.K. 1991. Phosphorus from internal sources in the Laurentian Great Lakes, and the concept of threshold external load. J. Great Lakes Res. 17: 132–140.

NÜRNBERG, G.K., R. HARTLEY, AND E. DAVIS. 1987. Hypolimnetic withdrawal in two North American lakes with anoxic phosphorus release from the sediment. Water Res. 21: 923–928.

O'MELIA, C.R. 1987. Particle–particle interactions, p. 385–404. *In* W. Stumm [ed.] Aquatic surface chemistry: chemical processes at the particle–water interface. John Wiley & Sons, Inc., New York, N.Y.

ONTARIO MINISTRY OF THE ENVIRONMENT. 1983. Handbook of analytical methods for environmental samples. Ontario Ministry of the Environment, Rexdale, Ont. 246 p.

PSENNER, R. 1988. Alkalinity generation in a soft-water lake: watershed and in-lake processes. Limnol. Oceanogr. 33: 1463–1475.

ROBINSON, R.B., F.J. DART, K. PISARCZYK, P.C. SINGER, AND W. SUNG. 1987. Committee report: research needs for the treatment of iron and manganese. J. Am. Water Works Assoc. 79(9): 119–122.

SCHEIDER, W.A., R.A. REID, B.A. LOCKE, AND L.D. SCOTT. 1983. Studies of lakes and watersheds in Muskoka–Haliburton, Ontario: methodology (1976–1982). Ont. Minist. Environ. Data Rep. DR 83/1. 1983.

SCHINDLER, D.W., R.W. NEWBURY, K.G. BEATY, AND P. CAMPBELL. 1976. Natural water and chemical budgets for a small Precambrian lake basin in Central Canada. J. Fish. Res. Board Can. 33: 2526–2543.

SCHNITZER, M., AND H. KERNDORFF. 1981. Reactions of fulvic acid with metal ions. Water Air Soil Pollut. 15: 97–108.

SHOLKOVITZ, E.R., AND D. COPLAND. 1982. The chemistry of suspended matter in Esthwaite Water, a biologically productive lake with seasonally anoxic hypolimnion. Geochim. Cosmochim. Acta 46: 393–410.

SIGG, L. 1987. Surface chemical aspects of the distribution and fate of metal ions in lakes, p. 319–350. *In* W. Stumm [ed.] Aquatic surface chemistry. Chemical processes at the particle–water interface. John Wiley & Sons, Inc., New York, N.Y.

SMITH, P.J. 1983. Sediment chemistry of lakes in the Muskoka–Haliburton study area. Ont. Minist. Environ. Data Rep. 83/7.

STAUFFER, R.E. 1986. Cycling of manganesee and iron in Lake Mendota, Wisconsin. Environ. Sci. Technol. 20: 449–457.

STUMM, W., AND J.J. MORGAN. 1981. Aquatic chemistry: an introduction emphasizing chemical equilibria in natural waters. John Wiley & Sons, Inc., New York, N.Y. 780 p.

SULZBERGER, B., J.L. SCHNOOR, R. GIOVANOLI, J.G. HERING, AND J. ZOBRIST. 1990. Biogeochemistry of iron in an acidic lake. Aquat. Sci. 52: 56–74.

TESSIER, A., F. RAPIN, AND R. CARIGNAN. 1985. Trace metals in oxic lake sediments: possible adsorption onto iron oxyhydroxides. Geochim. Cosmochim. Acta 49: 183–194.

URBAN, N.R., E. GORHAM, J. UNDERWOOD, F.B. MARTIN, AND J.G. OGDEN III. 1990. Geochemical processes controlling concentrations of Al, Fe, and Mn in Nova Scotia lakes. Limnol. Oceanogr. 35: 1516–1534.

VOLLENWEIDER, R.A. 1968. Water management research. DAS/CSI/68.27. Organization for Economic Co-operation and Development, Paris.

WAITE, T.D., AND F.M.M. MOREL. 1984. Photoreductive dissolution of colloidal iron oxide: effect of citrate. J. Colloid Interface Sci. 102: 121.

YAN, N.D., AND P.J. DILLON. 1984. Experimental neutralization of lakes near Sudbury, Ontario, p. 417–456. *In* J. Nriagu [ed.] Environmental impacts of smelters. John Wiley & Sons, Inc., New York, N.Y.

YAN, N.D., AND G.E. MILLER. 1984. Effects of deposition of acids and metals on chemistry and biology of lakes near Sudbury, Ontario, p. 243–282. *In* J. Nriagu [ed.] Environmental impacts of smelters. John Wiley & Sons, Inc., New York, N.Y.

Sequence of Sockeye Salmon Type 1 and 2 Growth Hormone Genes and the Relationship of Rainbow Trout with Atlantic and Pacific Salmon

Robert H. Devlin

Department of Fisheries and Oceans, 4160 Marine Drive, West Vancouver, BC V7V 1N6, Canada

Devlin, R.H. 1993. Sequence of sockeye salmon type 1 and 2 growth hormone genes and the relationship of rainbow trout with Atlantic and Pacific salmon. Can. J. Fish. Aquat. Sci. 50: 1738–1748.

Two types of growth hormone genes have been isolated from sockeye salmon (*Oncorhynchus nerka*) and their complete nucleotide sequence determined. The genes encode proteins of 210 amino acids and show considerable similarity to growth hormones characterized in other salmonids and fishes. The two genes presumably arose from a gene duplication event that generated the tetraploid condition in salmonids and are highly conserved in their coding regions. The sequences have diverged approximately 18% in noncoding regions since the gene duplication event and show numerous deletions and/or insertions. Isolation of these two genes from a Pacific salmon allows comparison of their sequences to growth hormone genes characterized from rainbow trout and from Atlantic salmon. The results indicate that rainbow trout is more similar to Pacific than to Atlantic salmon and suggest that Atlantic salmon diverged from Pacific salmonids at a time when sockeye and rainbow trout were part of a common breeding population. These results support the recent reclassification of rainbow trout from the genus *Salmo* to *Oncorhynchus*.

Deux types de gènes de l'hormone de croissance ont été isolés chez le saumon sockeye (*Oncorhynchus nerka*), et on en a déterminé la séquence complète des nucléotides. Les gènes codent des protéines de 210 acides aminés et présentent une ressemblance considérable avec les hormones de croissance caractérisées chez d'autres salmonidés et poissons. On pense que les deux gènes proviennent d'une duplication génétique qui a provoqué la tétraploïdie chez les salmonidés et ils sont fortement conservés dans leurs régions codantes. Les séquences ont divergé d'environ 18 % dans les régions non codantes depuis la duplication génique, et elles présentent de nombreuses délétions ou insertions. L'isolement de ces deux gènes chez un saumon du Pacifique permet de comparer leurs séquences aux gènes des hormones de croissance caractérisés chez la truite arc-en-ciel et le saumon de l'Atlantique. Les résultats montrent que cette truite ressemble davantage au saumon du Pacifique qu'à celui de l'Atlantique et ils portent à croire que ce dernier s'est séparé des salmonidés du Pacifique à une époque où le sockeye et l'arc-en-ciel faisaient partie d'une population commune. Ces résultats appuient la modification récente de la classification de la truite arc-en-ciel qui a été transportée du genre *Salmo* au genre *Oncorhynchus*.

Received October 9, 1992
Accepted March 5, 1993
(JB651)

Reçu le 9 octobre 1992
Accepté le 5 mars 1993

Growth hormone, or somatotropin, has been implicated in salmonids in the regulation of a number of physiological processes such as somatic growth and adaptation to seawater environments (see McLean and Donaldson 1992). Treatment of salmonids with growth hormone (GH) can result in tremendous gains in growth performance and feed conversion efficiencies, and consequently, much interest has been focused on the characterization of these hormones for application to aquaculture and fisheries enhancement. Isolation of cDNAs encoding somatotropin from salmonids has confirmed the existence of two forms of this hormone (Agellon et al. 1988a, 1988b; Rentier-Delrue et al. 1989a) presumably arising as a consequence of a genome duplication event (Ohno 1970) that occurred in ancestral salmonids some 25–100 million years ago (Allendorf and Thorgaard 1984). To date, the nuclear genes encoding the type 2 GH from rainbow trout (Agellon and Chen 1986) and Atlantic salmon (Johansen et al. 1989) have been characterized, as well as the type 1 gene from Atlantic salmon (Male et al. 1992). The isolation and characterization of GH sequences from salmonids has provided information relating to their structure and function, has allowed expression in nonhomologous systems to produce GH protein, and has allowed the production of transgenic fish with improved growth performance.

The classification of Pacific trouts has been controversial, particularly with regard to their potential ancestral relationship with either the Pacific salmon (*Oncorhynchus*) or Atlantic salmon (*Salmo*) genera (Regan 1914; Tchernavin 1939; Neave 1958). The classification of rainbow (*O. mykiss*) and cutthroat trout (*O. clarki*) has been recently reevaluated such that the Pacific trouts and salmon are now included within the genus *Oncorhynchus*, distinct from the genus *Salmo* containing Atlantic salmon (*S. salar*) and brown trout (*S. trutta*). This nomenclature change, now widely adopted, was made on the basis of morphological, biochemical, and mitochondrial DNA structure data (Smith and Stearley 1989). The present study provides the DNA sequence structure from both forms of GH genes present in the sockeye Pacific salmon (*Oncorhynchus nerka*). The characterization of these genes from a Pacific salmon enables a comparison of GH genes present in rainbow

trout with homologous sequences in Atlantic and Pacific salmon and allows a determination of phylogenetic relationships based on the DNA sequence of coding and noncoding regions within the nuclear genome.

Materials and Methods

Library Construction and Screening, Subcloning, and Sequencing

DNA was isolated (Devlin et al. 1991) from the liver of a single male sockeye salmon obtained from Weaver Creek in southwestern British Columbia in October 1989. A sockeye salmon genomic-DNA library was constructed in the phage vector lambda FIX (Stratagene Corp., La Jolla, Calif.) using instructions provided by the manufacturer. The library was plated and transferred to nitrocellulose filters (Sambrook et al. 1989) and GH-containing phage were identified with both the polymerase chain reaction (primers designed from consensus salmonid GH sequences) and a trout cDNA for GH (Agellon and Chen 1986). The primers used were as follows: GH1, 5′-GTCAGGATCCCATCCTTGGCAATTAAGAGT-3′; GH2, 5′-GTCAGGATCCCATCCTTGGCAATTAAGAGA- 3′; GH3, 5′- GTCAGAATTCACTGAACTCTTCTGAGTCTC-3′; GH4, 5′-GTCAGAATTCACCGCGATGTTGAAGAGCCG-3′; GH5, 5′-GTCAGGATCCAGCCTGGATGACAATGACTC- 3′; GH6, 5′- GTCAGAATTCCTACAGAGTGCAGTTGGCCT-3′. From positive phage, appropriate restriction fragments were subcloned into Bluescript II phagemid vectors (Stratagene Corp.) and a series of deletions and oligonucleotides were constructed to allow DNA sequencing of both strands of DNA from the putative GH clones. Sequencing was performed using a Sequenase kit (United States Biochemical Corp.) and was analyzed with the program PCGene (Intelligenetics).

DNA Sequence Analysis

Sequence similarities were identified using either the program NALIGN or CLUSTAL within the PCGene software package. Phylogenetic relationships were inferred from unweighted pair group mean analysis (UPGMA; Swofford and Olson 1990) performed on genetic distance data (percent sequence divergence) and from the program DNABOOT within the software package PHYLIP 3.4 provided by J. Felsenstein (Department of Genetics, University of Washington, Seattle, Wash.). This latter program performs a bootstrap analysis using an unrooted parsimony method of analysis to allow an estimation of the statistical validity of the phylogenetic relationships obtained (Felsenstein 1985). Relationships were only considered significant if they were supported in greater than 95% of the bootstrap replicates. Presentations of bootstrap tree data consist of unrooted trees and therefore only indicate internal relationships rather than ancestral groups.

Growth hormone sequence data utilized in this study were obtained from the following sources: chum type 1 cDNA (Sekine et al. 1985); rainbow trout type 1 and 2 cDNAs (Rentier-Delrue et al. 1989a) and type 2 gene (Agellon et al. 1988a); chinook type 1 cDNA (Hew et al. 1989); coho type 1 cDNA (Gonzalez-Villasenor et al. 1988); Atlantic salmon type 1 cDNA and type 1 gene share identical sequences (Lorens et al. 1989; Male et al. 1992) and type 2 gene (Johansen et al. 1989); carp cDNA (Chao et al. 1989); tuna (Sato et al. 1988); yellowtail (Watahiki et al. 1988); tilapia (Rentier-Delrue et al. 1989b); grass carp (Ho et al. 1989); red seabream (Momota et al. 1988); flounder (Watihiki et al. 1989); eel (Yamaguchi et al. 1990). Noncoding gene sequences data consisted of all introns combined with the terminator region from the stop codon to the poly-A addition signal.

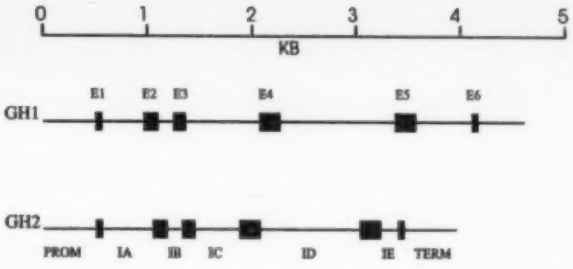


FIG. 1. Structure of type 1 and 2 GH genes from sockeye salmon. Exons are labelled E1–E6, introns are labelled IA–IE, and promoter and terminator regions are also shown. Size is indicated in kilobase pairs (KB).

Results

Structure and Organization of the Sockeye Growth Hormone Genes

Of 7×10^5 plaques from a sockeye salmon genomic DNA library that were screened, 17 retested positive for GH sequence and were purified for further analysis. Restriction mapping of these clones revealed the presence of two structurally different classes of genes (data not shown). Since the purpose of this work was to isolate both types of GH genes present in sockeye salmon, the polymerase chain reaction was used to identify those positive clones with homology to oligonucleotides designed from GH genes previously cloned from other salmonids. This approach also allowed the identification of two types of full-length genes which differed in the length of fragment amplified between exons 5 and 6. One clone from each of these two gene types was selected for sequence analysis.

A series of deletions and subclones were constructed from each of the two GH gene types to provide starting points for sequence analysis from different positions within each gene. Where appropriate deletions were not available, the sequence was extended from oligonucleotides that were designed from the sequence obtained in adjacent regions. From this analysis, it became clear that two structurally distinct types of GH genes had been isolated with homology to the type 1 and 2 cDNAs (Rentier-Delrue et al. 1989a). These two genes were named *OnGH1* and *OnGH2* ("On" abbreviating *Oncorhynchus nerka*), and their overall structure and complete nucleotide sequences are shown in Figs. 1 and 2.

Alignment of the GH gene sequences with cDNA sequences obtained from other salmonids allowed identification of six exons and five introns in both genes (Figs. 1 and 2). The sizes of the exons did not differ between the type 1 and 2 genes of sockeye salmon and were identical in length to those described for other salmonid GH genes (Agellon and Chen 1988a; Johansen et al. 1989; Male et al. 1992). The intron sizes differ both between type 1 and 2 genes of sockeye and among the three type 2 genes isolated to date (Table 1), and intron E from the sockeye type 1 gene contains an approximately 350 base pair insertion relative to the type 2 and the Atlantic type 1 genes. All

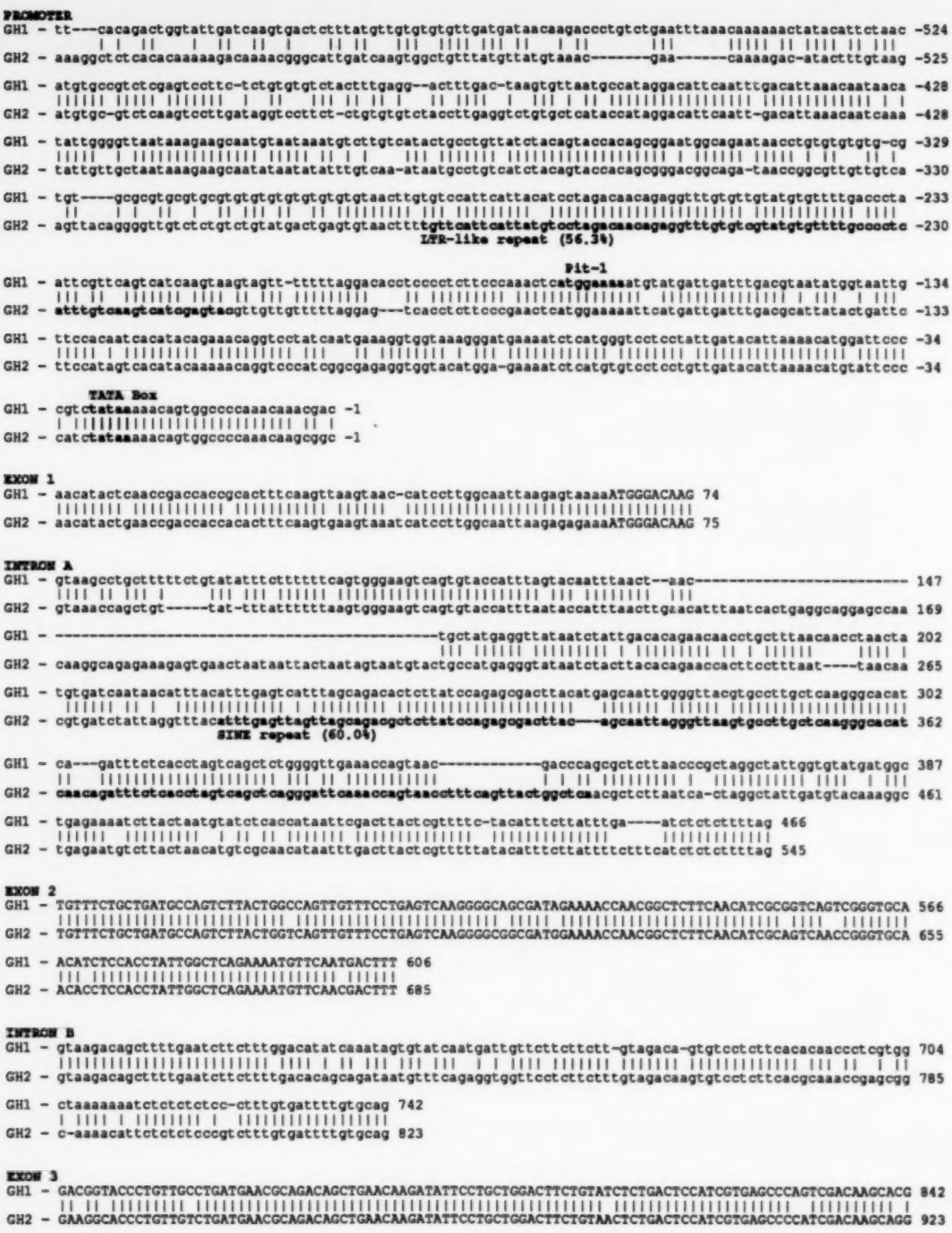


FIG. 2. Nucleotide sequence of type 1 and 2 GH genes from sockeye salmon. The sequences for the two genes are shown in an aligned format (derived from the NALIGN program within PCGene), with the nucleotide position shown at the right margin of each line starting at +1 for the putative transcription start site. The sequences are broken up into promoter, exon, intron, and terminator regions, with protein coding regions in uppercase letters and noncoding regions in lowercase letters. Identical nucleotides between the genes are separated by a vertical line, and gaps inserted in the sequence to improve the alignment are shown by dashes. Features such as transcription signals and homologies to various repetitive elements are shown in bold. (*Fig. 2 continued next page*)

FIG. 2. (*Continued next page*)

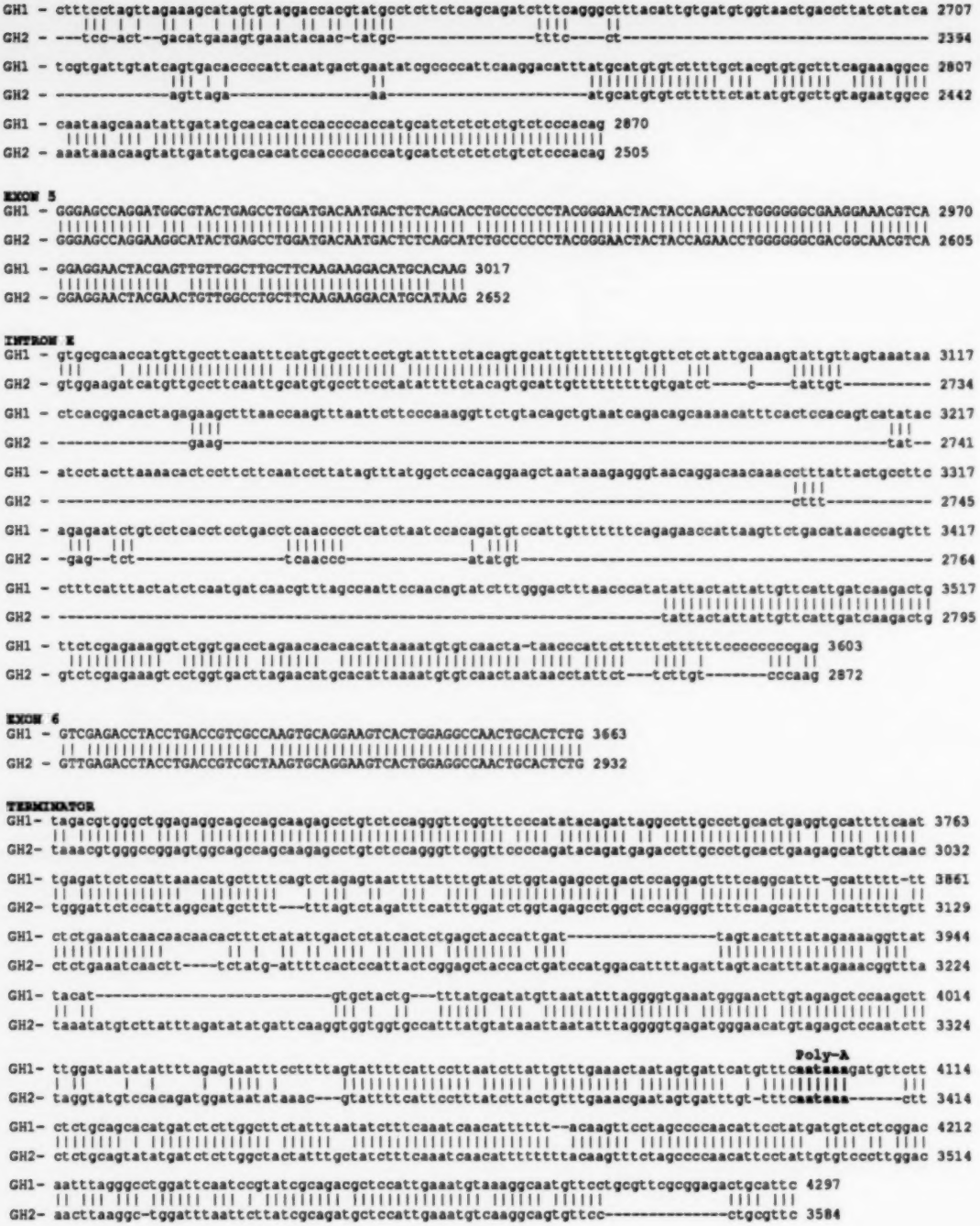


FIG. 2. (*Concluded*)

TABLE 1. Intron sizes (base pairs) of five GH genes from three salmonid species.

GH gene	Intron A	B	C	D	E	Terminator	Total
Sockeye1	392	136	710	1145	586	457	3426
Atlantic1	464	141	820	1116	218	483	3242
Sockeye2	470	138	445	964	220	488	2725
Atlantic2	461	136	461	1176	220	509	2963
Rainbow2	485	138	443	1115	245	508	2934
Homologous noncoding regions	385	141	401	931	91	435	1546

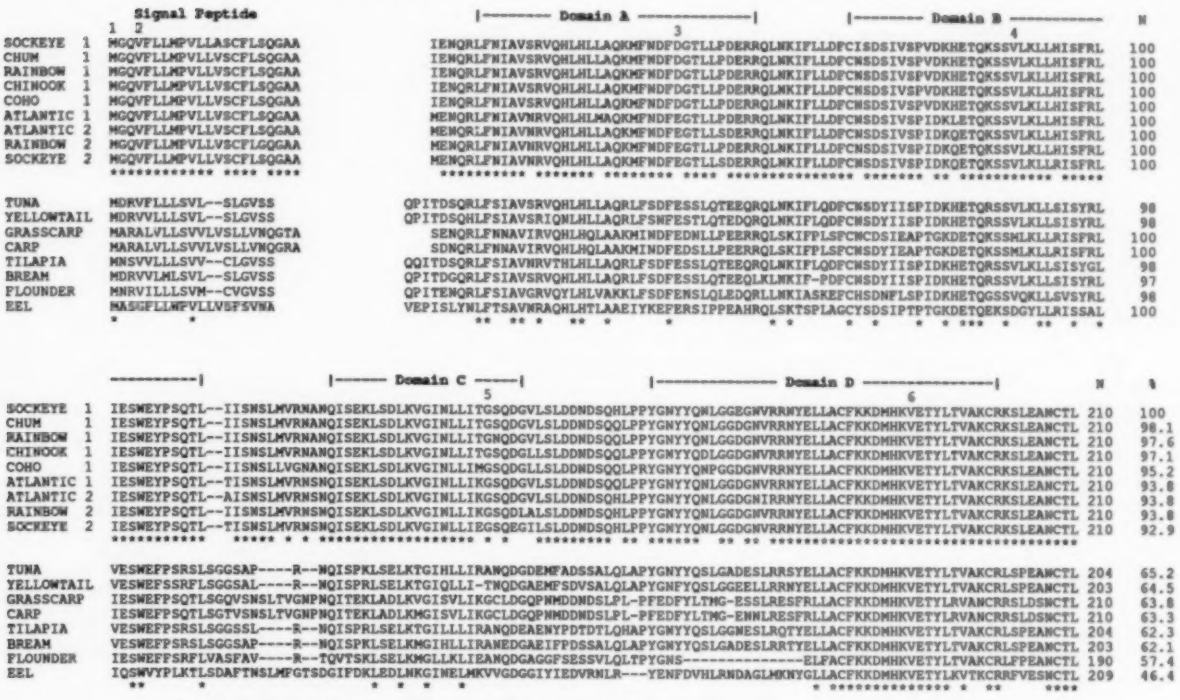


FIG. 3. Amino acid sequences derived from sockeye GH genes are shown aligned with GHs from other salmonids and fishes. Putative signal peptides are shown, as well as the four helical domain regions of these proteins. N represents the amino acid number starting from the N-terminus of the unprocessed peptide. Similarities (%) are expressed relative to the sockeye salmon type 1 protein, and an asterisk indicates those amino acids that are conserved among all proteins displayed above the asterisk. Numbers 1–6 above the protein sequence indicate the start position of the six exons in the salmonid genes.

intron–exon boundaries are flanked by the appropriate splice consensus, /GT...AG/, found in many other eukaryotic systems. The overall nucleotide lengths of these two genes from the promoter TATA box to the poly-A addition signal (see below) are 4138 for *OnGH1* and 3440 for *OnGH2*. This difference in size is largely due to multiple deletion and/or insertion events present in all introns except intron B. Transcriptional regulatory sequences could be identified in the 5′ flanking regions of both genes (shown in Fig. 2), including a TATA box and a putative Pit-1 (GHF-1) transcription factor binding site (Faisst and Meyer 1992). The putative transcription start site (nucleotide +1) is based on primer extension results reported for the trout GH gene by Agellon et al. (1988b) resulting in a relatively short untranslated 5′ leader sequence.

The base composition (percent A + T) from the TATA box to the poly-A addition signal in noncoding regions is 61.7 and 62.9% for the type 1 and type 2 genes, respectively, and 47.3 and 46.9% for coding regions from the same genes. Comparison of type 1 and 2 GH genes revealed a transition/transversion ratio of 2.67 (24/9) in coding regions. However, in the introns plus terminator regions, this ratio was only 0.79, with transitions (145) observed less frequently than transversions (183). A search of these GH gene sequences with repetitive elements previously characterized in fish (Matsumoto et al. 1986; Moir and Dixon 1988) revealed moderate homology in a number of locations as indicated in Fig. 2.

The protein coding regions of each gene were conceptually translated to predict the amino acid sequences shown in Fig. 3. Both genes encode 210 aa proteins with high similarity to other salmonid GH sequences. The mature forms of sockeye GH protein have been purified and analyzed to yield the sequence of the first 25 amino acids (Nagler et al. 1991). These protein

sequences correspond exactly to those present in the sockeye genes described in this study (see Fig. 3) and are consistent with assignment of Ile and Met as the amino terminal residues in the type 1 and 2 mature peptides, respectively. The mature proteins are both 188 amino acids in length, with predicted molecular weights of 21 510 daltons for the type 1 protein and 21 630 daltons for the type 2 protein. These weights are very close to those determined for purified sockeye GH protein by Nagler et al. (1991).

The predicted secondary structures of the sockeye GH proteins are similar to those described for other proteins in the GH family. From the crystal structure of porcine GH, Abdel-Meguid et al. (1987) have shown that four major helical domains can be found and that these are separated by turns and extended conformational regions. Figure 3 shows a similar arrangement observed in the salmonid sequences based on sequence conservation (see Kawauchi and Yasuda 1989) and that each domain is separated by at least one helix-turning proline residue. The most conserved regions among piscine GHs are found in domain D located at the COOH-terminal region of the protein. This region is partially buried inside the protein and has been implicated as being important in specifying tertiary conformational properties rather than as an integral part of the receptor binding site. The hydrophilic ends of domain D appear on the protein's surface as depicted by Abdel-Meguid et al. (1987), and this domain's hydrophobic middle is preceded by a region composed largely of extended conformations that may be involved in maintaining a stable uncharged internal structure. This latter region is the most poorly conserved among both salmonids and other fish species. The three other helical domain regions are well conserved among salmonids, although only domains A and B are moderately conserved among other fish species examined. Both types of sockeye GH possess 22 amino acid leader peptides that are strongly hydrophilic, a characteristic typical of other eukaryotic secretion peptides. Four cystine residues are present in both sockeye GH proteins that would allow the formation of the two disulfide bridges common to other proteins in this family.

Sequence Comparisons with Other Species

Comparison of the sockeye salmon GH protein sequences with different fish species (Fig. 3) indicates that all salmonid type 1 and 2 hormones characterized to date are very similar in structure (greater than 90%).

Figure 4A shows a UPGMA cluster dendrogram of DNA sequence similarities of the GH coding regions (630 bases) from several salmonids and carp. This analysis found separate monophyletic origins for type 1 genes and for type 2 genes, a result expected for duplicate genes that have evolved independently. This analysis also clustered rainbow trout with the other Pacific salmonids rather than with Atlantic salmon for both types of GH genes analyzed. Utilization of a bootstrapped parsimony analysis (DNABOOT program of PHYLIP 3.4 package) suggests (Fig. 4B) that the type 1 and 2 genes are indeed of separate lineages (hypothesis accepted 100 times out of 100, $p < 0.01$). However, the relationships among the different Pacific salmon species were not found to be significant, and the monophyletic origin of rainbow trout and Pacific salmon is significant (accepted 99 times per 100 runs) only when the type 1 sequences are examined and was not supported for the type 2 sequences.

To extend the analysis to a larger sequence data set, the relationships among coding and noncoding (introns and terminators) regions of five salmonid GH genes characterized to date are

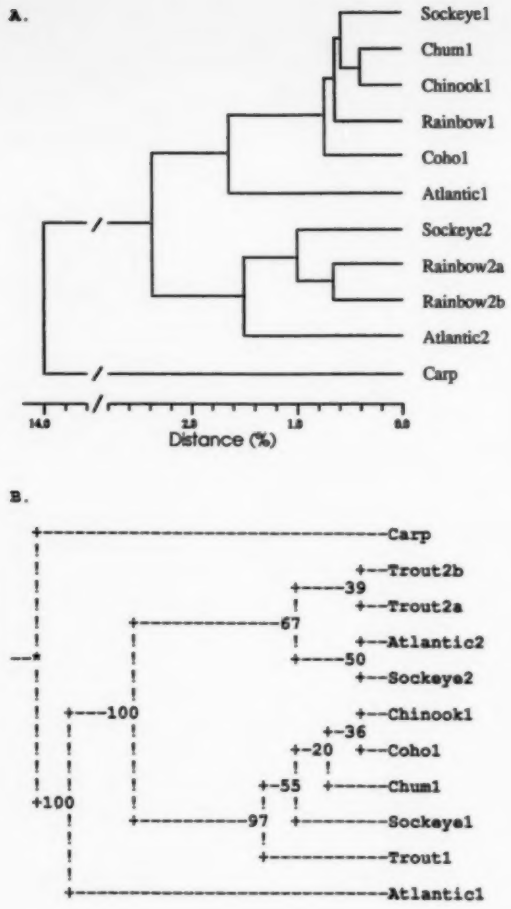


FIG. 4. Dendrograms of nucleotide sequence from coding regions of salmonid GH genes and cDNAs relative to carp sequence. (A) UPGMA cluster analysis. The distance between two species is determined by the total branch length between them, for example, Rainbow2a (gene) and Rainbow2b (cDNA) are approximately 1.2% divergent. (B) Parsimony analysis using PHYLIP 3.4. Numbers at the branch points indicate the number of bootstrap replicates out of 100 that supported this particular branch arrangement.

shown in Table 2. A UPGMA cluster analysis of noncoding regions is shown in Fig. 5A. This analysis again revealed the separation of type 1 from type 2 genes and showed that Atlantic and Pacific salmon have similar levels of sequence difference for both type 1 (13.5%) and type 2 (11.7%) genes and that divergence rates between the genera are consistent for the two gene types. This analysis also clustered rainbow trout and sockeye salmon as a monophyletic group distinct from Atlantic salmon. Bootstrap parsimony analysis indicated that this conclusion is very significant, grouping rainbow trout with the sockeye Pacific salmon 1000 out of 1000 times (Fig. 5B). An identical result is obtained using either the sockeye or Atlantic type 1 genes as the outgroup for analysis (not shown).

The type 1 gene from Atlantic salmon is more similar than the sockeye type 1 gene in comparisons with any of the three type 2 genes (Table 2). This difference largely arises from a major

TABLE 2. Percent similarity of salmon GH sequences. Noncoding regions (all introns and terminators) are above the diagonal and coding regions are below the diagonal. Parenthetical values are the sum of deletion and insertion events between the species.

	Sockeye1	Atlantic1	Sockeye2	Rainbow2	Atlantic2
Sockeye1	—	86.5	81.9	81.4	78.7
		(45)	(102)	(96)	(114)
Atlantic1	96.5	—	85.4	85.1	84.2
			(92)	(70)	(78)
Sockeye2	94.8	96.0	—	96.2	88.3
				(24)	(43)
Rainbow2	95.7	97.0	98.1	—	87.4
					(48)
Atlantic2	94.8	96.4	97.0	97.1	—

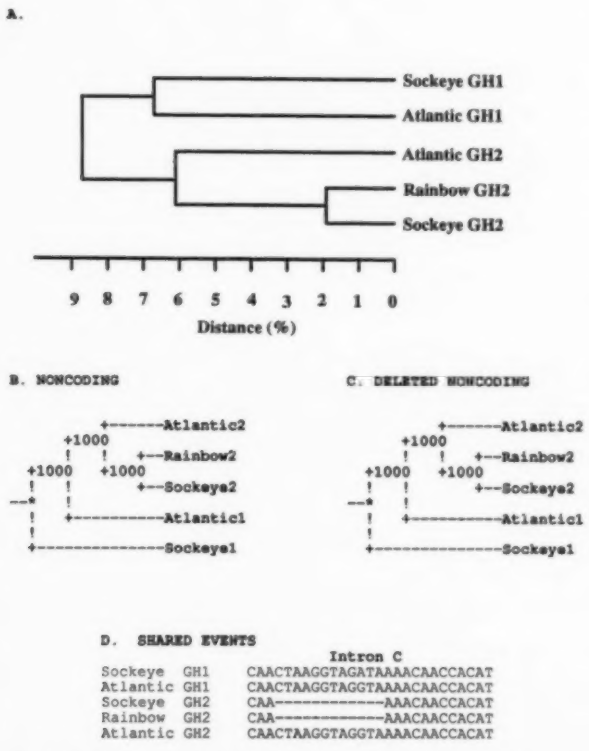


FIG. 5. Relationship of noncoding DNA regions among five salmonid species. (A) UPGMA analysis with distances shown as total branch lengths between species. (B) Parsimony analysis of noncoding regions (introns and terminator) for 1000 bootstrap replicates. (C) As in Fig. 5B but using noncoding sequence with deletion/insertion regions equalized to two base pairs in size. (D) Nucleotide sequence from the start of intron C showing shared deletion and substitution events found only in rainbow trout and sockeye salmon type 2 genes.

insertion present in the sockeye intron E relative to the other genes and shows how particular evolutionary events can have pronounced effects on the observed relationships between genes. The parsimony analysis utilized weighed each nucleotide position within a deletion or insertion region as a separate event (when in fact, most deletions or insertions represent single events of varying size), and therefore, an analysis was performed on sequences where this bias had been removed. All regions that had a deletion greater than three bases in length in one or more of the genes were reduced to a common size of two bases, producing homologous noncoding regions (Table 1) consisting of sequence blocks with more equal weighting of base substitutions and deletion/insertion events for analysis. Thus, this procedure effectively removes sequences that have unknown ancestry and limits similarity comparisons only to regions that are evolutionarily homologous. A bootstrap parsimony analysis (Fig. 5C) of these data again strongly supports a close relationship between rainbow trout and sockeye salmon distinct from the Atlantic salmon lineage (1000 out of 1000 times).

The relationship between these species is also suggested by the pattern of rearrangements that is observed among the genes. Some deletion events are located within regions that can be identified as ancestrally homologous between the type 1 and 2 genes, and their appearance in more than one species implies a common ancestry. For example (Fig. 5D), in intron C, a deletion/substitution event is found both in sockeye type 2 and rainbow type 2 genes at a location that is otherwise highly conserved between Atlantic type 2 and both Atlantic and sockeye type 1 genes. Presumably, this conserved sequence represents that of the ancestral GH gene before the tetraploidization event occurred, and the deletion event shared by sockeye salmon and rainbow trout indicates that they were historically part of a common breeding population distinct from Atlantic salmon. While several such deletion events within homologous regions support the rainbow trout as a Pacific salmon, none are present that suggest a close relationship of rainbow trout with Atlantic salmon. Several such deletion events are found in the vicinity of sequence differences that are shared only within either type 1 or type 2 genes, making it less likely that this pattern of shared rearrangements arose by gene conversion events between the two gene types.

Relationship between Type 1 and 2 Genes

The molecular sequence data show that a single type of gene is more alike between species than are type 1 and 2 genes within a species (Table 2), suggesting that disomic inheritance had largely been established by the time *Salmo* and *Oncorhynchus* diverged. However, the evidence available does not preclude the possibility that past exchange events (although perhaps recently occurring only rarely) occurred between type 1 and 2 genes. For example, while all *Oncorhynchus* type 1 mature peptides start with an Ile residue and all type 2 mature peptides start with a Met, in Atlantic salmon both types of GH begin with a Met

residue. Although these changes could be caused by mutation, they are also consistent with historical exchange events having occurred between the two types of GH genes in Atlantic salmon after divergence of *Salmo* and *Oncorhynchus* had occurred. To examine the extent to which sequences belonging to the ancestral type 1 and 2 GH genes may have intermixed within the different salmonid species, subsets of noncoding regions from the entire length of the genes (individual introns or terminators derived from the data set used in Fig. 5) were examined for their relationships by parsimony analysis (Fig. 6). This analysis shows that, although the phylogenetic relationships clearly observed for the larger data set were not always confirmed, the separation of type 1 and 2 genes was always maintained (100 out of 100 in all cases). Therefore, the two GH genes have undergone sufficiently little exchange that they can be unambiguously distinguished and that they probably represent loci that are undergoing largely independent evolutionary divergence. The clear separation to type 1 and 2 genes also strengthens the above phylogenetic conclusions derived from the comparison among type 2 genes, since such relationships must be deduced only from comparisons of orthologous genes.

Discussion

The family of proteins containing GH, prolactin, placental lactogen, and somatolactin (Ono et al. 1990) have been analyzed in a variety of vertebrates. It is believed that gene duplication events over the past 350 million years (Miller and Eberhardt 1983) have given rise to these functionally distinct hormones that influence growth and reproductive physiologies. The teleost GHs are approximatly 35–40% similar to their mammalian counterparts, yet many higher vertebrate GH proteins can have dramatic effects on growth performance in fish (McLean and Donaldson 1992).

During the course of salmonid evolution, a polyploidization event occurred some 25–100 million years ago that produced tetraploid organisms which ultimately gave rise to all present-day salmonids (Ohno 1970; Allendorf and Thorgaard 1984). This event effectively duplicated most or all genes in the proto-salmonid species and is a condition that is reflected in the current genome sizes (Hinegardner 1976) and karyotypes of these species (Simon 1964; Hartley 1987). Duplication of genetic loci may produce functional redundancies that could allow mutational damage to eliminate or change gene structures in the absence of strong selective pressures. Consequently, only approximately 50% of the salmonid genes now remain in a duplicated form (Allendorf 1978), the remainder presumably having been inactivated or eliminated during salmonid evolution. The present study has shown that two distinct genes for GH are present in sockeye salmon and that both have intact structures expected for transcriptionally active genes. A high degree of conservation is observed between the coding regions of these two genes, suggesting that functional constraints have operated to conserve the two proteins. Noncoding regions (introns and terminators) are, as expected, less well conserved between the two GH genes, showing many deletion/insertion events as well as base substitutions. It is interesting that an additional GH2 gene is located on the Y chromosome of some salmonid species (Du et al. 1993), suggesting that further duplication of GH loci also occurred.

Growth hormone genes contain five exons and four introns in all cases examined except for salmonids where there is a fifth

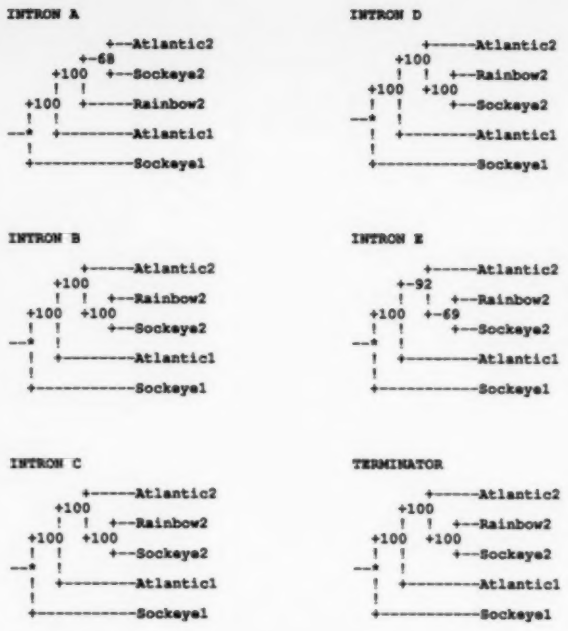


FIG. 6. Dendrograms from bootstrap parsimony analysis (100 replicates) of individual intron regions from salmonid growth hormone genes.

intron separating exons 5 and 6. This organization has been observed in type 1 and 2 GH genes in Atlantic salmon (Johansen et al. 1989; Male et al. 1992), in the type 2 gene of rainbow trout (Aggelon et al. 1988a), and in the present study for both the type 1 and 2 GH genes from sockeye salmon. The presence of the additional intron in both types of GH genes in these species indicates that it existed prior to the origin of salmonids. This intron is not found in the carp GH gene (Chiou et al. 1990), suggesting either that intron 5 was lost in carp and all other vertebrates after the salmonid lineage had split away or that salmonids acquired this intron after diverging from a carp–salmonid common ancestor.

The tetraploidization event presumably created two identical sets of homologous chromosomes. Initially, these two sets of chromosomes probably could associate randomly during meiosis, resulting in genetic exchange between homologous chromosomes as long as minimal differences in sequence and chromosome organization existed. Indeed, some vestiges of this nonhomologous pairing can still be observed in salmonid meiosis (May et al. 1982). Under these conditions, we would expect that duplicated genes would not be easily distinguished, since they would be exchanging portions of their coding and noncoding regions by recombination and gene conversion. At some point, however, sufficient mutational damage may have arisen such that effective pairing and exchange were no longer possible, resulting in two distinct chromosomal loci with disomic inheritance. Our analysis has shown that the noncoding regions in the type 1 and 2 GH genes of salmon are sufficiently different that they can be distinguished throughout their lengths. This suggests that little genetic exchange now occurs between the two genes and is consistent with the two GH genes located at distinct genetic loci.

The phylogenetic relationship of rainbow trout relative to Pacific and Atlantic salmon has been debated for many years (Smith and Stearley 1989). Rainbow trout was originally placed in the genus *Salmo* together with other salmonids including Atlantic salmon. However, rainbow trout was left in this genus when *Oncorhynchus* was adopted for Pacific salmon because rainbow trout have anal fin ray numbers more like those of Atlantic salmon and because some members of both rainbow trout and Atlantic salmon do not die after spawning, as well as other morphological and ecological characteristics. Many of these traits have subsequently not been found to be universally applicable, and other morphological and biochemical analyses (Regan 1914; Behnke 1970; Utter et al. 1973; Thomas et al. 1986; Thomas and Beckenbach 1989; McVeigh and Davidson 1991; Phillips and Pleyte 1991) have suggested a close link between rainbow trout and Pacific salmon. A reorganization of salmonid nomenclature has now been adopted, placing rainbow trout (*Oncorhynchus mykiss*) with other Pacific drainage salmon and trout rather than within the genus *Salmo* (Kendall 1988).

Insufficient differences exist in the coding regions of different salmonid GH genes to allow generation of reliable phylogenetic relationships (by parsimony analysis) for all salmonid species available. However, for rainbow trout, Atlantic salmon, and Pacific (sockeye) salmon, the type 2 gene has been completely sequenced (Agellon et al. 1988a; Johansen et al. 1989; this study) and provides considerable sequence information. In particular, noncoding intron regions from these genes provide sufficient variable sequence data to allow a significant determination, at the nuclear DNA level, of the relationship among GH sequences in these species. The results clearly showed that the rainbow trout gene was more closely related to the Pacific salmon gene than to the Atlantic salmon gene. In addition, deletions could be identified (based on comparisons of type 1 and 2 GH genes) in the intronic regions of rainbow trout that were shared with sockeye salmon alone, but no analogous rearrangements are shared between rainbow trout and Atlantic salmon. These results suggest that rainbow trout and sockeye Pacific salmon were members of a common breeding population at a time when Atlantic salmon had already begun a separate lineage and support the nomenclature change of rainbow trout from *Salmo gairdneri* to *Oncorhynchus mykiss*.

The rate of evolution (accumulation of sequence differences over time) of DNA sequences can be calculated if a time of divergence can be estimated. An estimate, utilizing available fossil and molecular data, places the origin of salmonids during the late Tertiary to early Cretaceous period between 25 and 100 million years ago (see Allendorf and Thorgaard 1984 for a discussion of the assumptions and weaknesses of this estimate). Between type 1 and 2 sockeye GH genes, 5.2% divergence is observed for coding regions and 18.1% for noncoding regions (16.2% for noncoding regions with the effect of deletion or insertion size removed) over this period. The average sequence divergence (including deletion/insertion events) between the noncoding regions of all five type 1 and 2 GH genes (Fig. 5A) is 17.2%. If 50 million years is used as an approximation for the genome duplication event, then these figures provide an estimate for the rate of sequence divergence (percent per million years) between the two types of sockeye salmon genes of from 0.10 (5.2/50) for coding regions to 0.36 (18.1/50) for noncoding regions. These values, however, could be in error, since the calculations assume that (1) the tetraploidization event coincided closely with the origin of salmonids and (2) the duplicated genes became distinct genetic loci soon thereafter. Since the sequence differences observed between the two genes only represent the changes that have accumulated since they began separate evolutionary lineages, the actual rates of mutation could be lower if tetraploidy arose before salmonids evolved, or higher if two distinct GH loci were not established until some time after. Thus, these estimates for GH genes in salmonids provide a crude estimate of the mutation rate and are only slightly lower than those observed in other organisms (Vawter and Brown 1986). Lower rates of evolution have been reported for mtDNA sequences in other cold-blooded vertebrates (Avise et al. 1992; Martin et al. 1992).

Examination of the level of sequence divergence between genes can also provide a relative estimate for the historical period during which Atlantic and Pacific salmon diverged. Atlantic salmon differ in their noncoding regions from sockeye salmon by 13.5% for the type 1 GH gene and 11.7% for the type 2 GH gene, an average of 12.6%. Assuming a constant molecular clock (and noting the concerns made above regarding tetraploidization, disomy, and the origin of salmonids), this value can be related to the average difference calculated above for type 1 and 2 GH genes (17.2%) and suggests that Atlantic salmon diverged from Pacific salmon after approximately one third of the time between the origin of the salmonids and the present (12.6%/17.2%). Similar calculations show that rainbow trout and sockeye salmon differ by only 3.8%, suggesting that they diverged more recently, approximately four fifths of the time after the origin of the salmonids. Although there is uncertainty regarding the origin of salmonids and the establishment of disomy after the tetraploidization event, and the potential of erroneous estimations arising from historical introgression of genetic information between species via hybridization (Smith 1992), these sequence relationships suggest that Pacific salmonids were undergoing speciation events during the Miocene, at least 11.0 million years ago (3.8/17.2 × 50 million years). This estimate is consistent with the characterization of Idahoan fossils from approximately 6 million years ago that resemble members of the present-day *Oncorhynchus* species (Smith 1992). The reciprocal calculations, utilizing 6 million years as the minimum estimate for the origin of Pacific salmonids, indicate that Pacific and Atlantic salmon diverged before 19.9 (12.6/3.8 × 6) million years ago and that the establishment of disomic inheritance after the tetraploidization event occurred before 27.2 (17.2/3.8 × 6) million years ago. This latter estimate is consistent with the existence of a protosalmonid fossil (*Eosalmo driftwoodensis*) recovered from the middle Eocene (Wilson 1977).

Acknowledgements

The author wishes to thank Ed Donaldson for his encouragement and Kelley Thomas, Eric Taylor, Don McPhail, Chan Woon-Khiong, Gary Wilson, Gerald Smith, and two reviewers for their helpful comments. The author also wishes to thank Dr. J. Felsenstein for making his set of phylogeny inference programs (PHYLIP 3.4) freely available to the scientific community

References

Abdel-Meguid, S.S., H. Shieh, W.W. Smith, H.E. Dayringer, B.N. Violand, and L.A. Bentle. 1987. Three dimensional structure of a genetically engineered variant of porcine growth hormone. Proc. Natl. Acad. Sci. USA 84: 6434–6437.

Agellon, L.B., and T.T. Chen. 1986. Rainbow trout growth hormone: molecular cloning of cDNA and expression in *Escherichia coli*. DNA

(N.Y.) 5: 463–471.

AGELLON, L.B., S.L. DAVIES, T.T. CHEN, AND D.A. POWERS. 1988a. Structure of a fish (rainbow trout) growth hormone gene and its evolutionary implications. Proc. Natl. Acad. Sci. USA 85: 5136–5140.

AGELLON, L.B., S.L. DAVIES, C.-M. LIN, T.T. CHEN, AND D.A. POWERS. 1988b. Rainbow trout has two genes for growth hormone. Mol. Reprod. Dev. 1: 11–17.

ALLENDORF, F.W. 1978. Protein polymorphism and the rate of loss of duplicate gene expression. Nature (Lond.) 272: 76–79.

ALLENDORF, F.W., AND G.H. THORGAARD. 1984. Tetraploidy and the evolution of salmonid fishes, p. 1–53. *In* B.J. Turner [ed.] The evolutionary genetics of fishes. Plenum Press, New York, N.Y.

AVISE, J.C., B.W. BOWEN, T. LAMB, A.B. MEYLAN, AND E. BERMINGHAM. 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the testudines. Mol. Biol. Evol. 9: 457–473.

BEHNKE, R.J. 1970. Phylogenetic problems in the family Salmonidae. Trans. Am. Fish. Soc. 1: 237–248.

CHAO, S., F. PAN, AND W. CHANG. 1989. Purification of carp growth hormone and cloning of the complementary DNA. Biochem. Biophys. Acta 1007: 233–236.

CHIOU, C., H. CHEN, AND W. CHANG. 1990. The complete nucleotide sequence of the growth-hormone gene from the common carp, *Cyprinus carpio*. Biochem. Biophys. Acta 1087: 91–94.

DEVLIN, R.H., B.K. MCNEIL, T.D.D. GROVES, AND E.M. DONALDSON. 1991. Isolation of a Y-chromosomal DNA probe capable of determining genetic sex in chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 48: 1606–1612.

DU, S.J., R.H. DEVLIN, AND C.L. HEW. 1993. Genomic structure of growth hormone genes in chinook salmon (*Oncorhynchus tshawytscha*): presence of two functional genes, GH-I and GH-II, and a male-specific pseudogene, GH-ψ. DNA Cell Biol. 12: 739–751.

FAISST, S., AND S. MEYER. 1992. Compilation of vertebrate encoded transcription factors. Nucleic Acids Res. 20: 3–26.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

GONZALEZ-VILLASENOR, L.I., P. ZHANG, T.T. CHEN, AND D.A. POWERS. 1988. Molecular cloning and sequencing of coho salmon growth hormone cDNA. Gene (Amst.) 65: 239–246.

HARTLEY, S. 1987. The chromosomes of salmonid fishes. Biol. Rev. 62: 197–414.

HEW, C.L., K.Y. TRINH, S.J. DU, AND S. SONG. 1989. Molecular cloning and expression of salmon pituitary hormones. Fish Physiol. Biochem. 7: 375–380.

HINEGARDNER, R. 1976. Evolution of genome size, p. 179–199. *In* F.J. Ayala [ed.] Molecular evolution. Sinauer Associates, Sunderland, Mass.

HO, W.K.K., W.H. TSANG, AND N.P. DIAS. 1989. Cloning of the grass carp growth hormone cDNA. Biochem. Biophys. Res. Commun 161: 1239–1243.

JOHANSEN, B., O.C. JOHNSEN, AND S. VALLA. 1989. The complete nucleotide sequence of the growth-hormone gene from Atlantic salmon (*Salmo salar*). Gene (Amst.) 77: 317–324.

KAWAUCHI, H., AND A. YASUDA. 1989. Evolutionary aspects of growth hormones from nonmammalian species, p. 51–68. *In* E.E. Mueller, D. Cocchi, and V. Locatelli [ed.] Advances in growth hormone and growth factor research. Springer-Verlag, Berlin.

KENDALL, R.L. 1988. Taxonomic changes in North American trout names. Prog. Fish-Cult. 50: 189.

LORENS, J., A.H. NERLAND, R. MALE, I. LOSSIUS, W. TELLE, AND G. TOTLAND. 1989. The nucleotide sequence of Atlantic salmon growth hormone cDNA. Nucleic Acids Res. 17: 2352.

MALE, R., A.H. NERLAND, J.B. LORENS, W. TELLE, I. LOSSIUS, AND G.K. TOTLAND. 1992. The complete nulceotide sequence of the Atlantic salmon growth hormone I gene. Biochem. Biophys. Acta 1130: 345–348.

MARTIN, A.P. G.J.P. NAYLOR, AND S.R. PALUMBI. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature (Lond.) 357: 153–155.

MATSUMOTO, K., K. MURAKAMI, AND N. OKADA. 1986. Gene for lysine $tRNA_1$ may be a progenitor of the highly repetitive and transcribable sequences present in the salmon genome. Proc. Natl. Acad. Sci. USA 83: 3156–3160.

MAY, B., J.E. WRIGHT, AND K.R. JOHNSON. 1982. Joint segregation of biochemical loci in Salmonidae. III. Linkage associations in Salmonidae including data from rainbow trout (*Salmo gairdneri*). Biochem. Genet. 20: 29–39.

MCLEAN, E., AND E.M. DONALDSON. 1992. The role of somatotropin in growth in poikilotherms, p. 43–71. *In* P.T.K. Pang and M.T. Schereibman [ed.] The endocrinology of growth, development, and metabolism in vertebrates. Academic Press Inc., New York, N.Y.

MCVEIGH, H.P., AND W.S. DAVIDSON. 1991. A salmonid phylogeny inferred from mitochondrial cytochrome *b* gene sequences. J. Fish Biol. 39: 277– 282.

MILLER, W.L., AND N.L. EBERHARDT. 1983. Structure and evolution of the growth hormone gene family. Endocrinol. Rev. 4: 97–130.

MOIR, R.D., AND G.H. DIXON. 1988. A repetitive DNA sequence in the salmonid fishes similar to a retroviral long terminal repeat. J. Mol. Evol. 27: 1–7.

MOMOTA, H., R. KOSUGI, H. HIRAMATSU, H. OHGAI, A. HARA, AND H. ISHIOKA. 1988. Nucleotide sequence of cDNA coding the pregrowth hormone of red seabream (*Pargus major*). Nucleic Acids Res. 16: 3107.

NAGLER, J.J., S.J. HWANG, AND D.R. IDLER. 1991. Isolation of sockeye salmon growth hormone utilizing serum triiodothyrine enhancement in rainbow trout to monitor biological activity. Gen. Comp. Endocrinol. 84: 374–380.

NEAVE, F. 1958. The origin and speciation of *Oncorhynchus*. Trans. R. Soc. Can. 52: 25–39.

OHNO, S. 1970. Evolution by gene duplication. Springer-Verlag, New York, N.Y.

ONO, M., Y. TAKAYAMA, M. RAND-WEAVER, S. SAKATA, T. YASUNAGA, T. NOSO, AND H. KAWAUCHI. 1990. cDNA cloning of somatolactin, a pituitary protein related to growth hormone and prolactin. Proc. Natl. Acad. Sci. USA 87: 4330–4334.

PHILLIPS, R.B., AND K.A. PLEYTE. 1991. Nuclear DNA and salmonid phylogenetics. J. Fish Biol. 39: 259–275.

REGAN, C.T. 1914. The systematic arrangement of the fishes of the family Salmonidae. Annu. Mag. Nat. Hist. 13: 405–408.

RENTIER-DELRUE, F., D. SWENNEN, L. MERCIER, M. LION, O. BENRUBI, AND J.A. MARTIAL. 1989a. Molecular cloning and characterization of two forms of trout growth hormone cDNA: expression and secretion of tGH-II by *Escherichia coli*. DNA (N.Y.) 8: 109–117.

RENTIER-DELRUE, F., D. SWENNEN, J.C. PHILIPPART, C. L'HOIR, O. BENRUBI, AND J.A. MARTIAL. 1989b. Tilapia growth hormone: molecular cloning of cDNA and expression in *Escherichia coli*. DNA (N.Y.) 8: 271–278.

SAMBROOK, J., E.F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning, a laboratory manual. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N.Y.

SATO, N., K. WATANABE, K. MURATA, M. SAKAGUCHI, Y. KARIYA, S. KIMURA, M. NONAKA, AND A. KIMURA. 1988. Molecular cloning and nucleotide sequence of tuna growth hormone. Biochim. Biophys. Acta 949: 35–42.

SEKINE, S., T. MIZUKAMI, T. NISHI, Y. KUWANA, A. SAITO, M. SATO, S. ITOH, AND H. KAWAUCHI. 1985. Cloning and expression of cDNA for salmon growth hormone in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 82: 4306–4310.

SIMON, R.C. 1964. Cytogenetics, relationships and evolution in Salmonidae. Ph.D. thesis, University of Washington, Seattle, Wash.

SMITH, G.R. 1992. Introgression in fishes: significance for paleontology, cladistics and evolutionary rates. Syst. Biol. 41: 41–57.

SMITH, G.R., AND R.F. STEARLEY. 1989. The classification and scientific names of rainbow and cutthroat trouts. Fisheries 14: 4–10.

SWOFFORD, D.L., AND G.J. OLSON. 1990. Phylogeny reconstruction, p. 411– 501. *In* D. Hillis and C. Moritz [ed.] Molecular systematics. Sinauer Associates, Sunderland, Mass.

TCHERNAVIN, V. 1939. The origin of salmon. Salmon Trout Mag. 95: 1–21.

THOMAS, W.K., AND A.T. BECKENBACH. 1989. Variation in salmonid mitochondrial DNA: evolutionary constraints and mechanisms of substitution. J. Mol. Evol. 29: 233–245.

THOMAS, W.K., R.E. WITHLER, AND A.T. BECKENBACH. 1986. Mitochondrial DNA analysis of Pacific salmonid evolution. Can. J. Zool. 64: 1058– 1064.

UTTER, F.M., F.W. ALLENDORF, AND H.O. HODGINS. 1973. Genetic variability and relationships in Pacific salmon and related trout based on protein variations. Syst. Zool. 22: 257–270.

VAWTER, L., AND W. BROWN. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rare variation in the molecular clock. Science (Wash., D.C.) 234: 194–196.

WATAHIKI, M., M. TANAKA, N. MASUDA, M. YAMAKAWA, Y. YONEDA, AND K. NAKASHIMA. 1988. cDNA cloning and primary structure of yellow tail (*Seriola quinqueradiata*) pregrowth hormone. Gen. Comp. Endocrinol. 70: 401–406.

WATAHIKI, M., M. YAMAMOTO, M. YAMAKAWA, M. TANAKA, AND K. NAKASHIMA. 1989. Conserved and unique amino acid residues in the domains of the growth hormones. J. Biol. Chem. 264: 312–316.

WILSON, M.V.H. 1977. Middle Eocene freshwater fishes from British Columbia. Life Sci. Contrib. R. Ont. Mus. 113: 1–61.

YAMAGUCHI, K., S. AKIKO, S. SEKINE, M. SATO, S. IHTO, K. SHIRAHATA, T. HIRANO, AND H. KAWAUCHI. 1990. Eel growth hormone. U.S. Patent Number: 4,894,362.

Harvesting Strategies and Fishing Mortality Reference Point Comparisons for the Northwest Atlantic Stock of Atlantic Mackerel (*Scomber scombrus*)

William J. Overholtz

National Marine Fisheries Service Woods Hole, MA 02543, USA

Overholtz, W.J. 1993. Harvesting strategies and fishing mortality reference point comparisons for the Northwest Atlantic stock of Atlantic mackerel (*Scomber scombrus*). Can. J. Fish. Aquat. Sci. 50: 1749–1756.

Previous research indicated that density dependence in population-level responses such as growth and predation mortality rate may play a major role in regulating the dynamics of the Northwest Atlantic stock of Atlantic mackerel (*Scomber scombrus*). Simulation studies, utilizing this compensatory model of the mackerel stock, suggest that expected yields and spawning stock sizes under conditions of constant harvest rate or constant catch would be quite disparate. Yields and spawning stock size would be less variable and slightly larger under a constant catch strategy for catches of mackerel up to approximately 200 000 t. However, a constant harvest rate strategy would provide higher long-term yields and a more stable spawning stock at catches greater than 200 000 t. A comparison of a constant catch policy with $F_{0.1}$, F_{20}, and F_{max} fishing strategies (constant F) suggests that the F_{max} and F_{20} strategies would cause a long-term decline in the spawning stock below the current minimum spawning stock target and provide highly variable yields. A constant catch strategy could produce nearly the same level of yield with considerably less variability and maintain a larger mean spawning stock biomass than the $F_{0.1}$ strategy.

Selon des travaux antérieurs de recherche, les phénomènes qui dépendent de la densité des populations, tels la croissance et le taux de mortalité due à la prédation, peuvent jouer un rôle majeur dans la régulation de la dynamique du maquereau de l'Atlantique (*Scomber scombrus*) appartenant au stock de l'Atlantique du nord-ouest. Des études de simulation à l'aide de ce modèle de compensation du stock de maquereaux portent à croire que le poids total des captures (rendement) et la taille du stock de géniteurs, prévus pour des taux constants de prélèvement ou une capture totale constante, seraient considérablement disparates. Le rendement et la taille du stock de géniteurs seraient moins variables et un peu plus grands si on se conformait à une stratégie de capture totale constante, jusqu'à concurrence de 200 000 t de maquereaux pêchés. Toutefois, une stratégie de taux constant de capture procurerait un rendement à long terme plus élevé et un stock de géniteurs plus stables si les captures dépassaient 200 000 t. D'après la comparaison d'une politique de capture totale constante et des stratégies de pêche $F_{0.1}$, F_{20} et F_{max} (F constant), les stratégies F_{max} et F_{20} provoqueraient un déclin à long terme du stock de géniteurs sous l'objectif minimal actuel et rendraient les rendements très variables. Une stratégie de capture totale constante aboutirait presque au même rendement, mais considérablement moins variable, et elle assurerait un stock de géniteurs dont la biomasse moyenne dépasserait celle qui découlerait de la stratégie $F_{0.1}$.

Received July 2, 1991
Accepted February 16, 1993
(JB108)

Reçu le 2 juillet 1991
Accepté le 16 février 1993

Atlantic mackerel (*Scomber scombrus*) have historically been extremely important to domestic fisheries in the United States and Canada and were the subject of a major distant-water fishery in the late 1960s and early 1970s in the mid-Atlantic region of the eastern United States and to a lesser degree in the Gulf of St. Lawrence (Anderson and Paciorkowski 1980). The stock is composed of two components that spawn in the Gulf of St. Lawrence and off the U.S. coast from New Jersey to Long Island, N.Y. (Sette 1950; Moores et al. 1975). From 1970 to 1976, landings averaged 310 000 metric tons (t), but the stock declined rapidly due to overexploitation by the distant-water fishery (Anderson and Paciorkowski 1980) (Fig. 1).

With the advent of the Magnuson Fishery Management and Conservation Act (MFCMA) in 1977, the responsibility for key fishery management decisions in the United States (including those that pertain to the mackerel stock) was transferred from the International Commission for Northwest Atlantic Fisheries (ICNAF) to the mid-Atlantic Fishery Management Council. In Canada, management responsibilities were delegated to the Atlantic Mackerel Advisory Committee.

In 1978, foreign catches of mackerel were reduced dramatically coincident with the collapse of the stock and a reduction of allocations to foreign countries by the Management Council. Total landings averaged about 33 000 t from 1979 to 1984 and have remained relatively low, averaging about 71 000 t since that time. The stock has recovered to levels comparable with those in the early 1970s due to low fishing mortality and the recruitment of the very large 1982 year class and several moderately large year classes (1981, 1985, 1986), but current forecasts of stock status are imprecise (Fig. 1) (Overholtz and Parry 1985; Anonymous 1987; Castonguay and Landry 1987; Overholtz 1991).

The present fishery management plan for the United States, developed by the Management Council in 1984, relies on annual catch quotas to manage Atlantic mackerel in the United States. It depends on an assessment of the stock based on a virtual

TABLE 1. Parameters used in the stochastic simulations of the Northwest Atlantic mackerel stock.

	Age (yr)	a	b	K
Weight (Wt_1)	1	0.122	-1.24×10^{-5}	—
Growth (G_i) increment	2	0.187	-1.42×10^{-5}	—
	3	0.154	-1.08×10^{-5}	—
	4	0.132	-1.03×10^{-5}	—
	5	0.102	-8.62×10^{-6}	—
Recruitment (R)	1	5.800	1.7	600.0
Maturity (%M)	2	0.543	-2.14×10^{-4}	—
	3	1.043	-2.14×10^{-4}	—
Predation mortality (M2)	1	0.600	3.00	2.5
	2	0.500	2.00	2.5

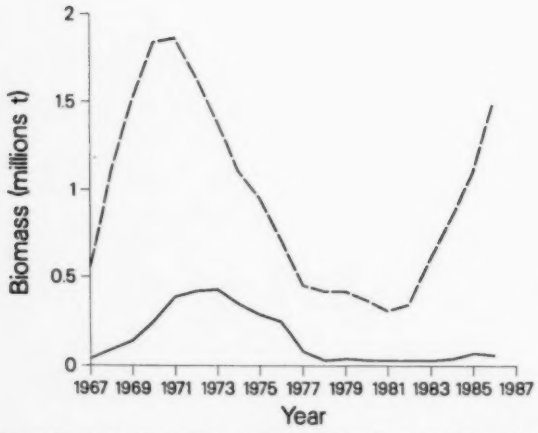


FIG. 1. Stock size (broken line) and annual landings (solid line) for the Northwest Atlantic mackerel stock for 1967–86.

population analysis (VPA) and an estimate of incoming recruitment at age 1 to provide projections of catch in the following year. Management objectives include maintenance of a minimum spawning stock biomass (SSB, 50% age 2, 100% age 3+) (600 000 t), annual quotas based on an $F_{0.1}$ catch strategy, and a subjective decision to maintain relatively low harvests to ensure the viability of an important spring recreational fishery.

The SSB minimum was determined subjectively from an examination of stock–recruit data by the Management Council. Likewise the annual quota is determined subjectively, after the Management Council examines available information each year. Current harvests from the fishery have averaged roughly 37 000 t since 1980 (Fig. 1) and have been considerably lower than annual estimates of $F_{0.1}$ catch (300 000+ t) would indicate are feasible (Overholtz and Parry 1985).

Several studies suggest that compensatory mechanisms may be important in regulating the dynamics of the Northwest Atlantic stock of Atlantic mackerel. Somatic growth rate in this stock is known to be dependent on abundance (Mckay 1979; Lett 1980; Overholtz 1989). Mackerel are also an important prey resource utilized by numerous predators; hence, natural mortality rates are likely higher than previous studies (Anderson 1982) have indicated (Overholtz et al. 1991a). Recent assessment advice for this stock was judged to be overly optimistic when compared with results from a model with density-dependent growth, sexual maturity, and predation mortality (Overholtz et al. 1991b).

Strategies for exploiting large pelagic fishery resources that exhibit a high degree of compensation are not well developed. Several studies have explored different policies for exploiting pelagic fishes (Swartzman et al. 1983; Armstrong 1984; Hall et al. 1988); however, an appropriate strategy for long-term exploitation is not evident at this time. Generally, constant catch strategies are thought to produce lower average catches and a higher probability of stock collapse than constant effort or F strategies (Murawski and Idoine 1989). Conversely, constant harvest rate strategies may produce high variability in yields (Armstrong 1984; Getz et al. 1987). Yields resulting from constant fishing patterns may be sufficiently variable to cause annual changes in landings and revenues that are unacceptable to fishermen or the fish processing sector (Armstrong 1984; Murawski and Idoine 1989).

The objective of this study was to compare constant catch and constant fishing mortality harvesting strategies for the Northwest Atlantic mackerel stock within a stochastic simulation modeling framework. The two approaches were examined by comparing model results for several performance and risk measures over a range of possible constant F and constant catch levels.

Model Background and Strategy

The feasibility of using a compensatory model to simulate the dynamics of this stock was investigated in another study that compared the current assessment methodology used for Atlantic mackerel with results from a model incorporating compensatory responses (Overholtz et al. 1991b). Results from that study suggest that a density-dependent model may represent stock dynamics in a more realistic fashion. Therefore, a compensatory model was used to simulate the dynamics of the Northwest Altantic mackerel stock in the current study. Overholtz et al. (1991b) have discussed the structure, assumptions, validation, and sensitivities of the model in detail, but a brief description of these elements of the approach is warranted here.

Growth

Differences in the distribution and life history of juvenile mackerel suggest that the initial pattern of growth in this species is unlike that experienced by older age groups (Overholtz 1989). Overholtz (1989) found that growth in this stock was significantly related to stock density for age groups 1–3 based on research survey data ($P \leq 0.066$) and age groups 1–5 based on commercial data ($P \leq 0.076$). He also found that large year classes grow very slowly ($P < 0.01$). Age-1 growth was modeled with the following relationship between weight at age 1 and year class size:

$$(1)\quad \mathrm{Wt}_1 = a - bN_1$$

where Wt_1 is the average weight (grams) at age 1, N_1 is year class size at age 1, and a and b are parameters (Table 1).

Growth in age groups 2–5 appears to be related to adult stock size (Overholtz 1989). Age 2–5 growth was determined by the relationship between weight in the previous year and weight gain in the present year with

$$(2)\quad \mathrm{Wt}_i = \mathrm{Wt}_{i-1} + G_i$$

where Wt_i is the average weight (grams) at age i, $i = 2, \ldots, 5$, Wt_{i-1} is the average weight from the previous year, and G_i is the annual growth increment (grams). The annual weight gain (Fig. 2A) for ages 2–5 is calculated from

$$(3)\quad G_i = a_i - b_i \mathrm{SS}$$

where SS is the adult stock size (numbers) and a and b are age-specific parameters (Table 1). Growth for the rest of the stock, 6+, was assumed to remain constant, following a pattern from the recent fishery (Overholtz 1989).

Sexual Maturity

Mackerel from this stock are all immature at age 1, fully mature at age 4, and exhibit varying degrees of sexual maturity at ages 2 and 3 (Overholtz et al. 1991b). An analysis of maturity data from 1981 to 1986 suggested that percent maturity at age 2 was probably negatively related to stock size, but at age 3, no consistent pattern was apparent. Percent maturity at age varied from 17 to 53% for age 2 and from 67 to 98% at age 3 (Overholtz et al. 1991b). These results are in general agreement with those of Moores et al. (1975). However, the Overholtz et al. (1991b) data set was of rather poor quality because it was not possible to collect an adequate number of samples during the period of peak maturation during 1981–86. Therefore, the null hypothesis of no effect of stock size on percent maturity at ages 2 and 3 could not be adequately tested with these data. However, since the Overholtz et al. (1991b) data indicated that the range in percent maturity for ages 2 and 3 was large, and changes in this mechanism may be important in the overall dynamics of the mackerel stock, the effect of compensatory responses in percent maturity on ages 2 and 3 was included in the current modeling study. Percent maturity (Fig. 2B) was estimated for ages 2 and 3 as

$$(4)\quad \%\mathrm{MAT}_i = a_i - b_i \mathrm{SSB}$$

where $\%\mathrm{MAT}_i$ is the percent maturity at age, SSB is the adult spawning stock biomass, and a and b are age-specific parameters (Table 1).

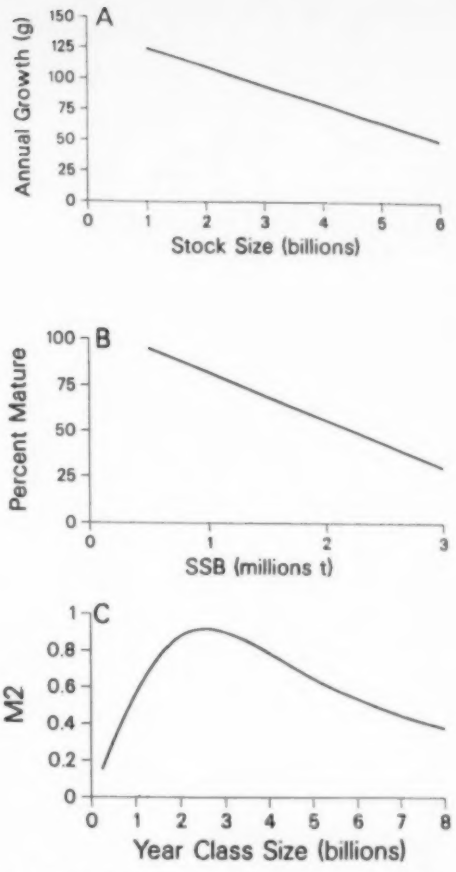


FIG. 2. Graphic representation of some of the relationships used in the compensatory model of the mackerel stock. (A) Annual growth increment for ages 2–5; (B) percent maturity for ages 2 and 3; (C) predation mortality rate for ages 1 and 2.

Predation Mortality

Predation on the younger age groups of mackerel is a significant source of mortality in this stock. Overholtz et al. (1991b) found that predation mortality (M2) is probably much higher than previously thought and primarily impacts age groups 1 and 2, based on the size distributions of mackerel found in the stomachs of predatory fish such as Atlantic cod (*Gadus morhua*), spiny dogfish (*Squalus acanthias*), and silver hake (*Merluccius bilinearis*). When predation mortality rates were calculated for a series of years with varying year class sizes, M2 appeared to be positively related to stock size (Overholtz et al. 1991b). Predation mortality was estimated with an equation relating M2 to year class size (Fig. 2C) with the relationship

$$(5)\quad \mathrm{M2}_i = (a \cdot \mathrm{SS}_i) / [1 + (\mathrm{SS}_i / K)^b]$$

where $\mathrm{M2}_i$ is the age-specific predation mortality rate, SS_i is the year class size at ages 1 and 2, and a, b, and K are age-specific parameters (Table 1). This relationship approximates a classical

TABLE 2. Values for mean catch, CV of catch, mean SSB, and the risk (P) of the SSB dropping below 600 000 t for constant harvest rate and constant catch strategies.

Fishing mortality	Mean catch	CV of catch	Mean SSB	P
		Constant harvest rate		
0.05	60 000	33.5	1170	0.002
0.10	100 000	35.1	1030	0.020
0.20	150 000	39.4	830	0.230
0.30	180 000	45.0	700	0.490
0.40	200 000	51.9	600	0.660
0.50	210 000	59.2	520	0.730
0.60	210 000	66.3	470	0.810
0.70	210 000	73.4	420	0.830
		Constant catch		
50 000	50 000	0.0	1200	0.003
100 000	100 000	0.0	1050	0.080
150 000	150 000	1.2	890	0.270
200 000	190 000	21.7	700	0.460
250 000	190 000	54.1	520	0.600
300 000	180 000	79.3	410	0.690
350 000	170 000	99.7	330	0.760
400 000	160 000	117.1	280	0.780

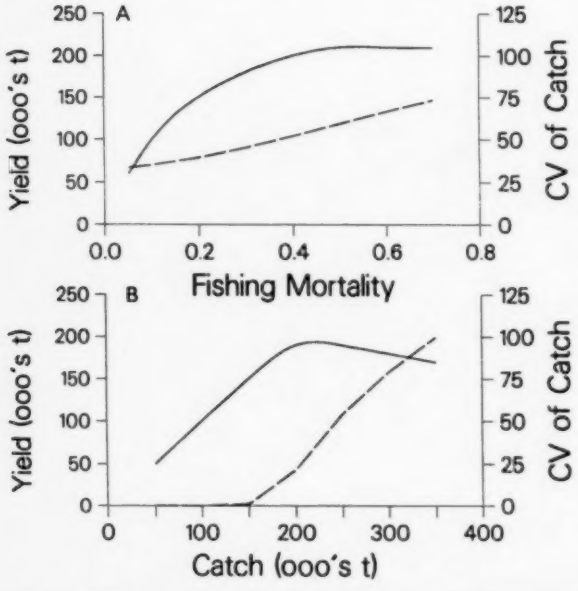


FIG. 3. Comparison of annual yield (solid line) and CV of yield (broken line) for (A) constant F and (B) constant catch harvesting strategies for the Northwest Atlantic mackerel stock.

type 3 predator response (Holling 1965; Murdoch 1973). It is consistent with the results of Overholtz et al. (1991b) and appears to be an appropriate predator–prey response model for fish (Murdoch 1973).

Recruitment

Recruitment at age 1 was estimated with a three-parameter stock recruitment model (Shepherd 1982) that utilized the variability in the stock–recruit data to produce stochastic estimates as

$$(6)\quad R = \{ (a \cdot \mathrm{SSB}) / [\, 1 + \mathrm{SSB}/K)^b \,] \} \cdot E$$

where R is the number of age-1 recruits, SSB is the spawning stock biomass, a, b, and K are parameters (Table 1), and E is a lognormally distributed $(1,S^2)$ random variable from the time series of mackerel stock–recruit data. The error term (E) changes with each iteration of the model, reflecting the variance in the stock–recruit data.

The model was fit using Shepherd's (1982) qualitative method and results in a mildly dome-shaped S/R curve. The procedure used for estimating the parameters of the error term utilized the residuals from the mackerel stock–recruit data to fit a white noise process (Sissenwine et al. 1988). These parameters were then used to generate a new multiplier for each model iteration.

Recruitment estimates were scaled upward by a constant factor to compensate for a bias in estimates of recruitment from VPA that are low because they do not include higher natural mortality rates due to predation (Harbowy 1989; Overholtz et al. 1991b). Work by Overholtz et al. (1991b) indicated that a multiplier of 1.5 was an appropriate factor because when the compensatory model, driven by historic F's, historic recruitment scaled by a factor of 1.5, and density-dependent growth, maturity, and predation mortality, was used to simulate historic (1967–85) landings and biomass, a close match to the historical pattern occurred. Trial simulations with this same model, without the recruitment multiplier, gave results that were inconsistent with the historic record because of high predation rates (Overholtz et al. 1991b).

Annual survival for 14 age groups was determined with a negative exponential relationship as

$$(7)\quad N_i = N_{i-1}\, e^{-Z}$$

where N_i is the number of survivors at age, N_{i-1} is the initial

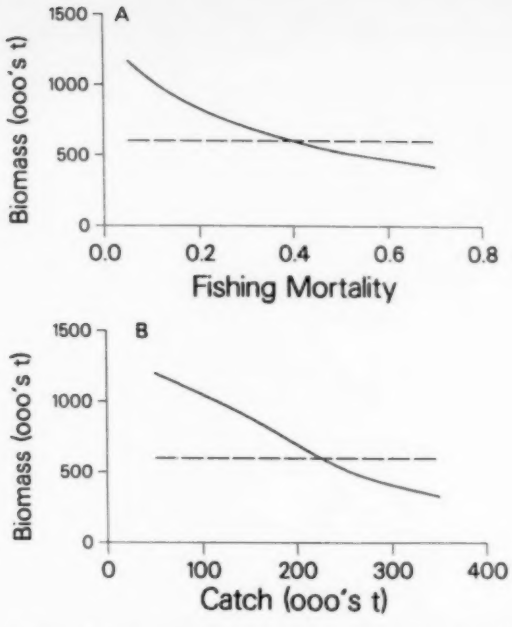


FIG. 4. Comparison of SSB (solid line) for (A) constant F and (B) constant catch harvesting strategies with the Management Council SSB minimum of 600 000 t (broken line) for the Northwest Atlantic mackerel stock.

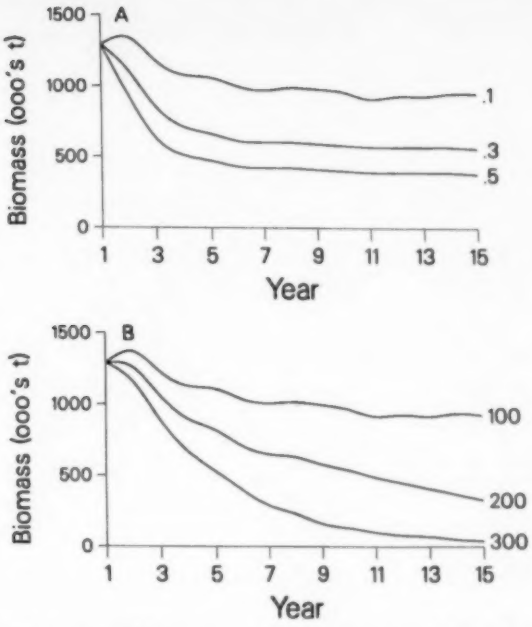


FIG. 5. Average SSB for three levels of (A) constant F (0.1, 0.3, 0.5) and (B) constant catch (110 000, 200 000, 300 000 t) for 15-yr simulations of the Northwest Atlantic mackerel stock.

number at age, and Z is the total instantaneous mortality rate.

Total mortality for the stock was assumed to be additive and of the form

$$(8)\quad Z_i = (\mathrm{M1} + \mathrm{M2}_i + F_i)$$

with M1 constant for all age groups at 0.2 and where $\mathrm{M2}_i$ is the predation mortality rate applied for age groups 1 and 2 only and F_i is the age-specific fishing mortality rate.

Annual yields from the stock were calculated with the Baranov catch equation as

$$(9)\quad C_i = N_i (F_i / Z_i)(1 - e^{-Z_i})$$

where C_i is the annual age-specific yield in number, N_i is the number at age, and $i = 1,\ldots,14$.

The model was used to compare constant harvest rate and constant catch strategies and investigate common fishery reference points for the mackerel stock. Simulations were conducted for fishing mortalities from 0.05 to 1.0 and for constant catches from 50 000 to 500 000 t. Model outputs were based on 500 Monte Carlo simulations of the system over a 15-yr time horizon.

Results

Harvest Rate versus Constant Catch Strategies

Yield

Expected average yield under a constant fishing mortality policy would increase asymptotically to about 215 000 t over the range of F studied (Fig. 3A). Coefficients of variation start out at roughly 30% and increase steadily to about 80% at an F of 0.7. Yields under a constant catch strategy increase linearly to a peak at about 190 000 t at the 200 000–250 000 t level of constant catch and then decline (Fig. 3B). Variability in average yield would be small until constant catches approach 200 000 t and then would increase rapidly to about 100% at 350 000 t, due to the averaging process involved in the Monte Carlo results (i.e., stock collapse occurs in some of the model runs) (Fig. 3B).

Initial yields would be less variable for the constant catch strategy up to roughly 200 000 t for comparable levels of average yield (i.e., $F = 0.35$, yield = 190 000 t and CC = 200 000 t, yield = 190 000 t) (Table 2; Fig. 3). Beyond this the constant F policy would result in higher, less variable yields.

Spawning stock biomass

The Management Council SSB threshold of 600 000 t was determined by subjective examination of the mackerel stock–recruit data, so in a sense it is arbitrary. However, it is currently written into the U.S. management plan and thereby serves as a useful reference biomass level. Mean SSB under the constant harvest rate strategy would decline to the Management Council threshold of 600 000 t at an F of 0.4 and gradually decrease after that (Fig. 4A). The spawning stock would decline similarly, although the response curve is shaped differently, at a constant catch of just beyond 200 000 t and then drop quickly after that (Fig. 4B). The initial decline in SSB appears to be more dramatic under the constant F strategy, while the decline in SSB at higher rates of exploitation would be faster under the constant catch strategy (Fig. 4).

Time trajectories for the two strategies under varying conditions of constant harvest rate and catch produce dramatically different patterns in spawning stock biomass. For comparative purposes, three levels for each strategy were chosen to represent each case (Fig. 5). After an initial drop, SSB

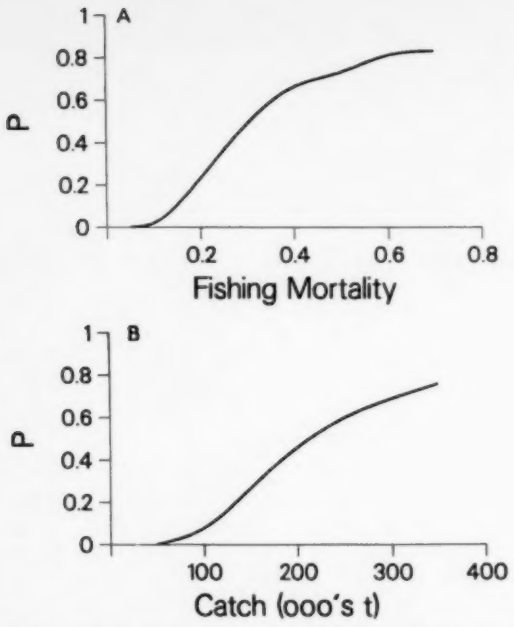


FIG. 6. Comparison of the probability of spawning stock failure (P) (declining below 600 000 t) for (A) constant F and (B) constant catch harvesting strategies for the Northwest Atlantic mackerel stock.

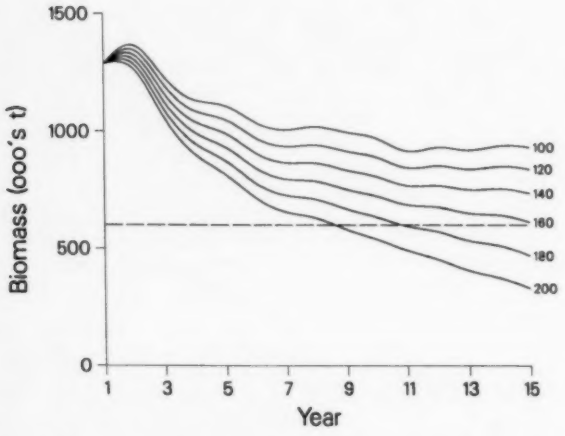


FIG. 7. Comparison of average SSB over a 15-yr period for constant catches from 100 000 to 200 000 t with the Management Council SSB minimum of 600 000 t (broken line).

appeared to stabilize for all the constant F trajectories (Fig. 5A). Constant catches of 100 000 t produced a 15-yr equilibrium, but SSB declined steadily for constant catches of 200 000 and 300 000 t (Fig. 5B).

Probability of SSB declining

The probability of the SSB dropping below 600 000 t, the Management Council threshold for this stock, was measured by calculating the ratio of failures to the number of possible years in the 15-yr time span at each level of constant F and catch. For

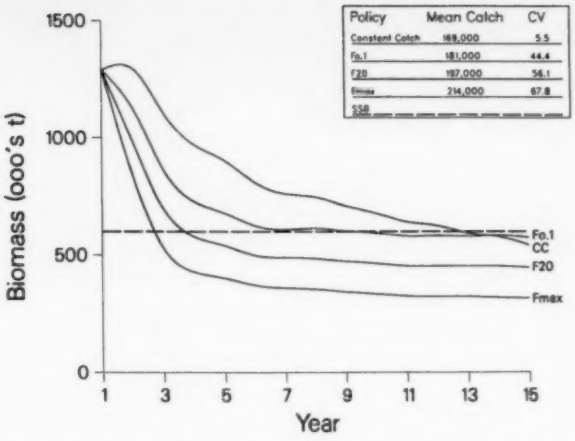


FIG. 8. Comparison of SSB for a constant catch of 170 000 t (CC), $F_{0.1}$, F_{20}, and F_{max} over a 15-yr period with the Management Council SSB minimum of 600 000 t (broken line); corresponding mean catches and CV of catches are presented.

comparable levels of catch the probability of failure was higher initially for the constant F strategy (Table 2; Fig. 6). This trend did not continue because the rate of failure increased steadily for the constant catch strategy but leveled off at about 0.8 for the harvest rate strategy (Fig. 6).

Sustainability

To determine an approximate level of sustainable yield for the mackerel stock, the constant catch model was run at catches from 100 000 to 200 000 t in increments of 20 000 t. Catches of 150 000–160 000 t could be sustained voer a 15-yr period while keeping the SSB above 600 000 t (Fig. 7). Catches of 130 000–140 000 t could probably be sustained over a longer period. Catches above 180 000 t would deplete the stock quickly, driving the SSB well below 600 000 t after several years (Fig. 7). These results agree with and enhance the yield and SSB projections for the constant catch strategy developed previously in Fig. 3B and 4B.

Constant Catch versus Common F Reference Points

A comparison of a constant catch strategy with three common constant F reference points (F_{max}, F_{20}, and $F_{0.1}$) suggests that in the long term, these strategies would produce widely different trends in yield and SSB. The F_{max} strategy would provide a high mean catch (214 000 t), but SSB would stabilize at just over 300 000 t, well below the minimum SSB level of 600 000 t (Fig. 8). At F_{20} (Mace and Sissenwine 1993), catches would average 197 000 t and SSB would be about 425 000 t. Fishing the stock at $F_{0.1}$ (Gulland and Boerema 1973) would result in a mean catch of 181 000 t and an SSB of roughly 575 000 t after 15 yr. A comparable constant catch scenario of 170 000 t would result in a mean catch of 169 000 t and a considerably larger SSB for most of the 15-yr period, dropping below 600 000 t in year 13 and thereafter (Fig. 8).

The CV's of the catches under these four options would be greatly different, with low variability for the constant catch strategy and progressively larger CV's for the $F_{0.1}$, F_{20}, and F_{max} strategies, respectively. For roughly the same level of average

yield, the constant catch strategy would produce a much more stable catch and larger SSB and the $F_{0.1}$ policy for nearly the entire 15 yr (Figure 8).

Discussion

A typical conclusion from harvest strategy studies is that a constant harvest rate policy is the more favorable approach for the long-term exploitation of a fishery resource (Murawski and Idoine 1989). This would be true if the principal goal of a management program were to obtain the largest cumulative catch and stock–recruit dynamics were stationary. A harvest rate policy, recognizing the inherent uncertainty in the estimates of yield and stock size that can be provided from such an approach, is an expensive process that will produce highly variable catches.

Under some circumstances, a constant catch strategy might be more practical for exploiting a fishery resource. A low level of constant catch could, of course, be sustained indefinitely for many species, but the biological and/or economic arguments of forgone yield are generally too compelling for this to be a reasonable approach to exploitation. However, the removal of a moderate level of constant catch may be a very reasonable harvest policy in some cases when stability of yield is a management goal (i.e., for sociopolitical reasons).

Harvesting strategies such as illustrated by the constant catch and fishing mortality (F) comparisons simulated in this study are worth considering when the best policy for the management of fishery resources is being considered. Apparently none of the reference point fisheries would protect the mackerel stock in terms of SSB declines below the Management Council minimum. However, removing 170 000 t annually would result in roughly the same level of catch, a higher average SSB, and much less variability in yield than exploiting the stock at the $F_{0.1}$ level. True, the expectation would be for the spawning stock to decline at some future date as a result of this policy, but this needs to be weighed against some of the potential disadvantages of the constant harvest rate strategy.

Mean annual yields under the $F_{0.1}$ strategy simulated for this stock would vary by ±44%, ranging from 100 000 to 260 000 t, and SSB would be reduced dramatically for the first several years after implementation. This much variability in yield is usually disruptive for the fishing industry, making budgeting and planning a difficult task. It would also require large annual shifts in total allowable catch (TAC), resulting in major fluctuations in the catch of individual vessels. Fishery managers and the fishing industry may be more willing to accept a constant annual catch with some loss in overall yield, rather than the large fluctuations associated with the process of setting highly uncertain catch quotas, especially for a low-valued species such as mackerel.

Managers and the industry may also appreciate the ease of monitoring a constant catch policy rather than attempting to track annual changes in effort or set annual TAC's. If SSB began to decline to unacceptably low levels after several years under a constant catch harvest policy, the reductions in catch necessary to stabilize the SSB would be much smaller than the annual fluctuations in yield experienced under a constant harvest rate strategy and would probably be more acceptable to the fishing industry.

Results suggest that both strategies may be useful depending on the goals and circumstances of a particular fishery. A reduction in effort to an optimal level would probably provide substantial biological and economic benefits for almost any fish stock and if achievable would probably be the best overall policy to pursue. However, many fisheries are complex, being prosecuted by multiple user groups with variable gear, vessel types, and economic goals. Under such circumstances, a constant catch policy may be quite practical for social and/or political reasons.

A constant catch policy might be particularly useful in the initial several years of exploitation or "fishing up" a stock, expecially if estimates of stock size are uncertain. Stock biomass would be reduced much more slowly, than under an $F_{0.1}$ or F_{20} strategy, for example, allowing for a more precise determination of stock size in future years. Exploitation of the stock could proceed at a controlled pace, allowing for the collection of new data and reestimation of stock sizes. For a species such as Atlantic mackerel with the potential for large compensatory stock responses and major uncertainties in providing assessment advice, a constant catch approach to exploitation would be particuarly beneficial.

References

ANONYMOUS. 1987. Status of the fishery resources off the Northeastern United States for 1987. NOAA Tech. Mem. NMFS-F/NEC-50. U.S. Department of Commerce, Woods Hole, Mass. 132 p.

ANDERSON, E.D. 1982. Status of the Northwest Atlantic mackerel stock — 1982. Ref. Doc. 82-44, Woods Hole Laboratory, Northeast Fisheries Center, National Marine Fisheries Service, NOAA, Woods Hole, Mass. 45 p.

ANDERSON, E.D., AND A.J. PACIORKOWSKI. 1980. A review of the Northwest Atlantic mackerel fishery. Rapp. P.-v. Réun. Cons. int. Explor. Mer 177: 175–211.

ARMSTRONG, M.J. 1984. An analysis of yield variability from three harvesting strategies in the South African anchovy fishery, under conditions of randomly fluctuating recruitment success. S. Afr. J. Mar. Sci. 2: 131–144.

CASTONGUAY, M., AND J. LANDRY. 1987. Le maquereau blue (*Scomber scombrus* Linne') du nord-ouest de l'Atlantique, sous-regions 2 a 6 de l'OPANO. Con. Sci. Cons. Peches Can. Atl. CSCPCA Doc. rech. 87/47. Institut Maurice Lamontagne, Mont-Joli, Quebec. 34 p.

GETZ, W.M., R.C. FRANCIS, AND G.L. SWARTZMAN. 1987. On managing variable marine fisheries. Can. J. Fish. Aquat. Sci. 44: 1370–1375.

GULLAND, J.A., AND L.K. BOEREMA. 1973. Scientific advice on catch levels. Fish. Bull. U.S. 71: 325–335.

HALL, D.L., R. HILBORN, M. STOCKER, AND C.J. WALTERS. 1988. Alternative strategies for Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 45: 888–897.

HARBOWY, J. 1989. A multispecies model of fish stocks in the Baltic sea. Dana 7: 23–43.

HOLLING, C.S. 1965. The functional response of predators to prey density and its role in mimicry and population regulation. Mem. Entomol. Soc. Can. 91: 293–320.

LETT, P.F. 1980. A comparative study of the recruitment mechanisms of cod and mackerel, their interaction, and its implication for duel stock assessment. Can. Tech. Rep. Fish. Aquat. Sci. 988: 45 p.

MACE, P.M., AND M.P. SISSENWINE. 1993. How much spawning per recruit is enough? *In* S.J. Smith, J.J. Hunt, and D. Rivard [ed.] Risk evaluation and biological reference points for fisheries management. Can. Spec. Publ. Fish. Aquat. Sci. 120.

MCKAY, K.T. 1979. Synopsis of biological data of the northern population of Atlantic mackerel (*Scomber scombrus*). Can. Fish. Mar. Serv. Tech. Rep. 885: 26 p.

MOORES, J.A., G.H. WINTERS, AND L.S. PARSONS. 1975. Migrations and biological characteristics of Atlantic mackerel (*Scomber scombrus*) occurring in Newfoundland waters. J. Fish. Res. Board Can. 32: 1347–1357.

MURAWSKI, S.A., AND J.S. IDOINE. 1989. Yield sustainability under constant-catch policy and stochastic recruitment. Tran. Am. Fish. Soc. 118: 349–367.

MURDOCH, W.W. 1973. The functional response of predators. J. Appl. Ecol. 10: 335–342.

OVERHOLTZ, W.J. 1989. Density-dependent growth in the Northwest Atlantic stock of Atlantic mackerel (*Scomber scombrus*). J. Northwest Atl. Fish. Sci. 9: 115–121.

OVERHOLTZ, W.J. 1991. Stock assessment of the Northwest Atlantic mackerel stock. Papers of the Northeast Regional Stock Assessment Workshops. Res. Doc. SAW 12/5, Northeast Fisheries Science Center, Woods Hole, Mass. 30 p.

OVERHOLTZ, W.J., S.A. MURAWSKI, AND K.L. FOSTER. 1991a. Impact of predatory fish, marine mammals, and seabirds on the pelagic fish ecosystem of the Northeastern USA. *In* N. Daan and M.P. Sissenwine [ed.] Symposium on Multispecies Models Relevant to Management of living resources. Rapp. P.-v. Réun. Cons. int. Explor. Mer 193: 198–208.

OVERHOLTZ, W.J., S.A. MURAWSKI, AND W.L. MICHAELS. 1991b. The impact of compensatory responses on assessment advice for the Northwest Atlantic mackerel stock. Fish. Bull. 89: 117–128.

OVERHOLTZ, W.J., AND B.L. PARRY. 1985. Update of the status of the Northwest Atlantic stock of Atlantic mackerel for 1985. Ref. Doc. 85–113, Woods Hole Laboratory, Northeast Fisheries Center, National Marine Fisheries Service, NOAA, Woods Hole, Mass. 5 p.

SETTE, O.E. 1950. Biology of the Atlantic mackerel (*Scomber scombrus*) of North America: Part 2: Migrations and habits. Fish. Bull. U.S. 51: 251–358.

SHEPHERD, J.G. 1982. A versatile new stock–recruitment relationship for fisheries and the construction of sustainable yield curves. J. Cons. int. Explor. Mer 40: 67–75.

SISSENWINE, M.P., M.J. FOGARTY, AND W.J. OVERHOLTZ. 1988. Some fisheries management implications of recruitment variability, p. 129–152. *In* J.A. Gulland [ed.] Fish population dynamics. 2nd ed. John Wiley & Sons, Inc., New York, N.Y.

SWARTZMAN, G.L., W.M. GETZ, R.C. GETZ, R.T. HARR, AND K. ROSE. 1983. A management analysis of the Pacific whiting (*Merluccius productus*) fishery using an age-structured stochastic recruitment model. Can. J. Fish. Aquat. Sci. 40: 524–539.

Comparison of Dynamic Models for Edible Phytoplankton

Stephen R. Carpenter

Center for Limnology, University of Wisconsin, Madison, WI 53706, USA

Richard C. Lathrop

Bureau of Research, Wisconsin Department of Natural Resources, 3911 Fish Hatchery Road, Fitchburg, WI 53711, USA

and Alejandro Muñoz-del-Rio

Department of Statistics and Center for Limnology, University of Wisconsin, Madison, WI 53706, USA

Carpenter, S.R., R.C. Lathrop, and A. Muñoz-del-Rio. 1993. Comparison of dynamic models for edible phytoplankton. Can. J. Fish. Aquat. Sci. 50: 1757–1767.

Alternative models for the dynamics of edible phytoplankton were compared using long-term data from mesotrophic Paul Lake and eutrophic Lake Mendota. Alternative models fit to the data contrasted linear versus logistic algal growth, type I versus type II functional response, and prey-dependent versus ratio-dependent predation. In both lakes, the model with lowest prediction error had logistic algal growth and a type I, prey-dependent functional response. Under these models, the spring bloom and clear-water phase of productive lakes can be explained as an incomplete predator–prey cycle: the spring pulse of edible algae is followed by a peak of zooplankton; then, edible algae are overgrazed leading to the clear-water phase and collapse of the grazer biomass. This study demonstrates the use of time series data at the ecosystem scale to identify process-based models, contrast alternative models on a probabilistic basis, and estimate parameters. This approach avoids the assumptions involved in extrapolating ecosystem models from smaller scale studies.

Différents modèles de la dynamique du phytoplancton comestible ont été comparés entre eux, au moyen de données collectées sur une longue période dans le lac Paul, mésotrophe, et le lac Mendota, eutrophe. Les modèles fidèles opposaient la croissance linéaire et logistique des algues, les réponses fonctionnelles de type I et de type II ainsi que la prédation en fonction du rapport du nombre de proies et la prédation en fonction du rapport du nombre de prédateurs au nombre de proies. Dans les deux lacs, le modèle prédictif le plus juste était de type I, de croissance logistique dépendant du nombre de proies. Selon ce type de modèle, les fleurs d'eau printanières et le stade de la clarification des eaux dans les lacs productifs peuvent s'expliquer par le caractère incomplet du cycle prédateurs–proies : la prolifération printanière des algues comestibles est suivie d'une augmentation du zooplancton; les algues comestibles sont ensuite surconcommées, ce qui mène à la clarification des eaux et à l'effondrement de la biomasse des herbivores. L'étude démontre l'emploi des séries chronologiques de données à l'échelle de l'écosystème pour identifier les modèles de processus, mettre en opposition différents modèles sur une base probabiliste et estimer les paramètres. On évite ainsi les hypothèses à poser dans l'extrapolation des modèles d'écosystèmes obtenus d'études à petite échelle.

Received August 7, 1992
Accepted February 16, 1993
(JB592)

Reçu le 7 août 1992
Accepté le 16 février 1993

In ecosystem modeling, the mathematical form of the interactions and the parameter values are often derived from small-scale controlled experiments. This approach has been effective for achieving some of the goals of ecosystem modeling. However, small-scale experiments and the models based on them sometimes lead to contrasting and confusing predictions of large-scale behavior. Additionally, this approach has the danger of including too much detail and the unknown errors introduced by estimating ecosystem-scale parameters in small-scale experiments (Walters 1986; Frost et al. 1988). These problems can be overcome by direct modeling of key interactions using time series data (Walters 1986; Hilborn and Walters 1992).

Lake plankton dynamics have been addressed by many models which differ in important ways. A fundamental question is the extent to which plankton fluctuations result from exogenous physical–chemical forcing or endogenous biotic oscillations (Murdoch and McCauley 1985; Carpenter and Kitchell 1987; Walters et al. 1987). Long-term records show that both processes influence phytoplankton (Jassby et al. 1990). In terms of ecological models, the answer to this question depends in part on the functional form of feedbacks among components of the planktonic system. A variety of equations, all justifiable on the basis of small-scale experiments, lead to such varied outcomes as stable equilibria, time-lag cycles, or predator–prey cycles (Rosenzweig 1971; Walters et al. 1987; McCauley and Murdoch 1990; Carpenter 1992). Fits of process-based models to time series can help evaluate these alternatives.

This paper focuses on the dynamics of edible phytoplankton in lakes. Fluctuations in edible phytoplankton are crucial to the "paradox of enrichment" (Rosenzweig 1971). Predator–prey cycles of edible phytoplankton and their grazers may facilitate dominance of nuisance blue-green algae (Carpenter 1992). In modeling dynamics of edible algae, we include the effects of grazers. However, an independent model of grazer dynamics is beyond the scope of this paper. Such a model would have to include life history delays (McCauley and Murdoch 1990) and

TABLE 1. Models for edible algal biomass dynamics compared in this study. $A(t)$ is edible algal biovolume at time t, $Z(t)$ is zooplankton biomass at time t, Δ_t is the duration of the time interval in days, and the c_i are parameters to be estimated statistically. The number used to refer to each model in the text, type of algal growth term (LIN = linear; LOG = logistic) type of functional response (type I or II), and form of the feedback from prey to predator (PD = prey dependent; RD = ratio dependent) are also presented.

Model	Algal growth	Functional response	Form	Equation for $A(t+1)=$
1	LIN	I	PD	$A(t) + c_1\Delta_t A(t) - c_3\Delta_t A(t) Z(t)$
2	LOG	I	PD	$A(t) + c_1\Delta_t A(t) - c_2\Delta_t A(t)^2 - c_3\Delta_t A(t)Z(t)$
3	LIN	II	PD	$A(t) + c_1\Delta_t A(t) - [c_3\Delta_t A(t) Z(t)/(c_4 + A(t))]$
4	LOG	II	PD	$A(t) + c_1\Delta_t A(t) - c_2\Delta_t A(t)^2 - [c_3\Delta_t A(t) Z(t)/(c_4 + A(t))]$
5	LIN	I	RD	$A(t) + c_5\Delta_t A(t)$
6	LOG	I	RD	$A(t) + c_5\Delta_t A(t) - c_2\Delta_t A(t)^2$
7	LIN	II	RD	$A(t) + c_1\Delta_t A(t) - [c_3\Delta_t A(t)/(c_4 + A(t)/Z(t))]$
8	LOG	II	RD	$A(t) + c_1\Delta_t A(t) - c_2\Delta_t A(t)^2 - [c_3\Delta_t A(t)/(c_4 + A(t)/Z(t))]$

effects of zooplanktivory (Rudstam et al. 1993).

According to the hypothesis of the paradox of enrichment, predator–prey oscillations of edible phytoplankton–grazer systems should increase in amplitude as lakes become more nutrient rich (Rosenzweig 1971). Large-amplitude fluctuations indeed occur in some models of plankton in eutrophic lakes (Vanni et al. 1992). In oligotrophic lakes, analyses of long-term data show that plankton dynamics are essentially equilibrial (Walters et al. 1987). Our objective is to examine time series data from more nutrient-rich lakes to determine whether oscillatory dynamics occur as predicted by the paradox of enrichment. The goal is to compare alternative models of algal dynamics (Hilborn and Walters 1992), an approach distinct from the more descriptive time series models of phytoplankton studied by Jassby et al. (1990), Lathrop and Carpenter (1992a, 1992b), and Rudstam et al. (1993). We examined 7 yr of data from mesotrophic Paul Lake and 15 yr of data from eutrophic Lake Mendota.

Comparison of Models

Eight models for edible phytoplankton biomass were compared (Table 1). Each model represents the effects of history and physical–chemical conditions through a term of the form $A(t) + c\Delta_t A(t)$ and the effects of grazing loss through a term that contains zooplankton biomass, $Z(t)$. Some of the models also include a logistic self-limitation term of the form $c_2\Delta_t A(t)^2$. The factor Δ_t is included in the models to allow for small differences in the time intervals between sampling points.

The models are of two different forms, depending on whether the functional response of grazing is proportional to prey concentration or to the ratio of prey to predator (Arditi and Ginzburg 1989; Hanski 1991). In the prey-dependent form (models 1–4), the functional response depends on prey concentration, but not on predator concentration (Hanski 1991). The feedback from prey to predator is assumed to occur through the numerical response of the predators (Hanski 1991). In the ratio-dependent form (models 5–8), the feedback from prey to predator is assumed to occur through competition among predators for prey (Arditi and Ginzburg 1989). Thus, the functional reponse depends on the ratio of prey to predators.

For both the prey-dependent and ratio-dependent groups of models, all four combinations of two prey growth terms and two functional response terms are considered (Table 1). Prey growth options are linear (models 1, 3, 5, and 7) or logistic (models 2, 4, 6, and 8). Models of planktonic ecosystems have used both linear (Carpenter and Kitchell 1987; Walters et al. 1987; Vanni et al. 1992) and logistic (Steele and Henderson 1981; Carpenter 1992) growth. Functional response options are type I (i.e., linear; models 1, 2, 5, and 6) or type II (i.e., asymptotic; models 3, 4, 7, and 8). Both type I (Walters et al. 1987; Elser and Goldman 1991) and type II (Steele and Henderson 1981; Porter et al. 1982; Carpenter and Kitchell 1987; Carpenter 1992; Vanni et al. 1992) functional responses have been used in models of planktonic ecosystems.

These models are derived and explained in ecological terms by Hassell (1978) and Arditi and Ginzburg (1989). In writing the models for statistical analysis (Table 1), we have combined parameters that are not separable in the statistical analysis of prey time series. For example, the ratio-dependent model with linear prey growth and type I functional response is

$$(1)\quad A(t+1) = A(t) + k_1\Delta_t A(t) - k_2\Delta_t[A(t)/Z(t)]Z(t)$$

where k_1 is the per capita prey growth rate and k_2 is the predation coefficient. This equation reduces to

$$(2)\quad A(t+1) = [1 + (k_1 - k_2)\Delta_t]A(t).$$

Clearly the parameters k_1 and k_2 cannot be separated by regression of $A(t+1)$ on $\Delta_t A(t)$. Consequently, we fit model 5 in Table 1, where the coefficient $c_5 = k_1 - k_2$. By a similar argument, in the models with logistic growth, we set $c_2 = r/K$, the ratio of the potential growth rate to the carrying capacity.

Methods

Limnological Data

Phytoplankton and zooplankton time series were obtained for two lakes of contrasting productivity. Paul Lake (near Land o' Lakes, Wisconsin, USA) is a stained, mesotrophic lake with modest total phosphorus (~15 $\mu g \cdot L^{-1}$) and chlorophyll *a* (~5 $\mu g \cdot L^{-1}$) concentrations in summer (Carpenter and Kitchell 1993). Lake mendota (in Madison, Wisconsin, USA) is eutrophic, with relatively high total phosphorus (~50$\mu g \cdot L^{-1}$) and chlorophyll *a* (~30 $\mu g \cdot L^{-1}$) concentrations in summer (Kitchell 1992).

Paul Lake was sampled weekly during the summer stratified seasons of 1984–90. The original data were presented by Carpenter and Kitchell (1993), so methods will be described only briefly here. Zooplankton were collected in vertical net hauls,

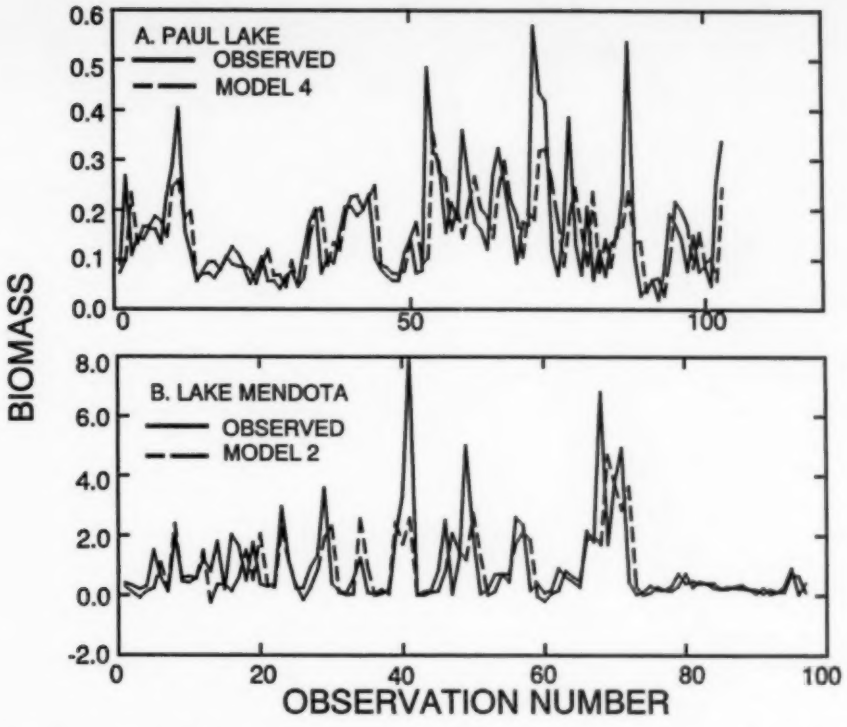


FIG. 1. Biovolume (mg wet mass·L^{-1}) of edible phytoplankton versus observation number in (A) Paul Lake and (B) Lake Mendota. Both observations and predictions of model 2 (Lake Mendota) or model 4 (Paul Lake) are shown. Each prediction is based on the previous observation. The time interval is usually 7 d in Paul Lake and 10–14 d in Lake Mendota. Raw data are tabulated in the Appendix.

preserved, and enumerated and measured by species. Biomasses were calculated using length–weight regressions. Net hauls were calibrated regularly against vertical profiles collected with a Schindler–Patalas trap. Phytoplankton were collected from three epilimnetic depths and pooled for analysis. Cells and colonies were enumerated and measured by taxon. Biovolumes (i.e., the volume of protoplasm exclusive of sheaths and extracellular structures) were calculated using geometric formulae.

Lake Mendota was sampled approximately biweekly during the ice-free seasons of 1976–90. Methods will be described only briefly here; for details, see Lathrop and Carpenter (1992a, 1992b). Zooplankton were collected in vertical net hauls, preserved, and enumerated and measured by species. Length–weight regressions were used to compute biomass. Net efficiency was checked by comparisons with a vertically integrated sample obtained with a 10-cm-diameter tube, which was lowered to depth and capped by a diver. Phytoplankton were collected in a composite epilimnetic sample, preserved in Lugol's solution, and enumerated under an inverted microscope. As in Paul Lake, biovolumes were computed using geometric formulae.

For fitting the dynamic models, we used time series of edible algal biovolume and *Daphnia* biomass. Following past studies in these lakes, we defined edible phytoplankton as those cells or colonies with biovolume less than that of a 30-μm-diameter sphere (Bergquist and Carpenter 1986; Lathrop and Carpenter 1992a). *Daphnia* account for most of the grazing in these lakes during summer stratification (Lanthrop and Carpenter 1992b; Carpenter and Kitchell 1993), so we used *Daphnia* biomass as a surrogate for total grazer biomass. In Paul Lake, we analyzed sequential data points collected 7 d apart. In Lake Mendota, we analyzed sequential data points collected 10–14 d apart.

Statistical Analysis

In comparing the models, our primary criterion was to choose the model that minimized residual error (Reilly 1970; Walters 1986). We also considered prediction error and the precision with which parameters could be estimated.

From a statistical standpoint, the complete system we wish to model is

$$A(t+1) = f[A(t), Z(t)] + \omega(t) \quad (3a)$$

$$A(t) = A_{obs}(t) + \nu_A(t) \quad (3b)$$

$$Z(t) = Z_{obs}(t) + \nu_Z(t) \quad (3c)$$

where f is one of the models in Table 1, A and Z are the true biomasses of edible algae and zooplankton, A_{obs} and Z_{obs} are the observed biomasses of edible algae and zooplankton, ω is process error, and ν_A and ν_Z are observation errors (Hilborn and Walters 1992). Process error refers to deviations of system state at time $t + 1$ from model projections based on the actual system state at time t. Process errors can arise from inadequacies of the model, or from external perturbations of the system. Observation

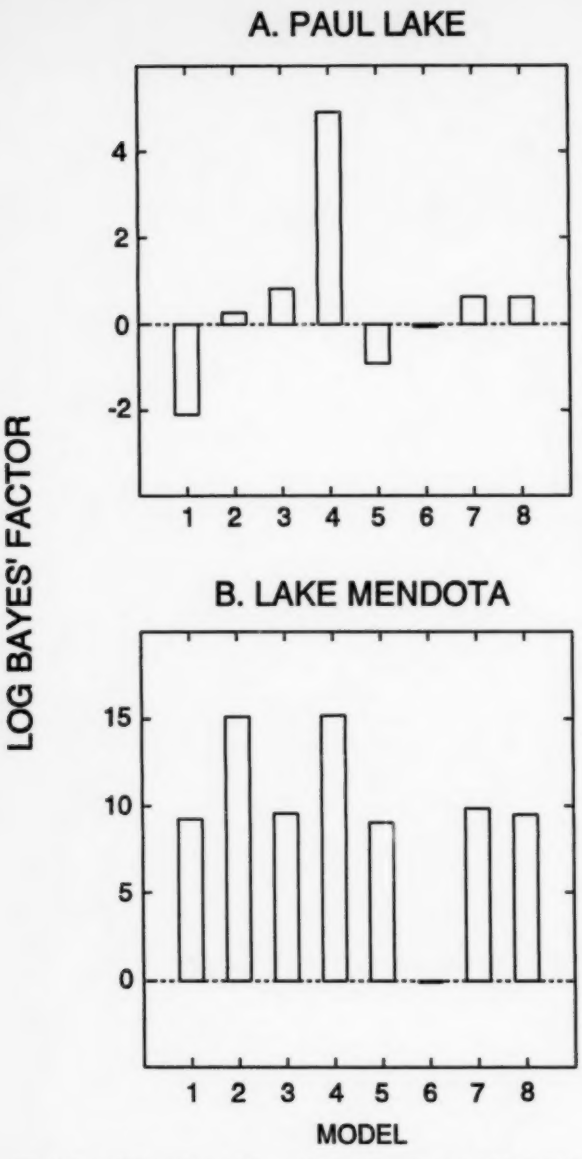


FIG. 2. Base 10 logarithm of Bayes' factor for each model (Table 1) in comparison with the simple alternative $A(t+1) = A(t)$. A positive log Bayes' factor indicates that the model is superior to the simple alternative. (A) Paul Lake; (B) Lake Mendota.

error refers to deviations of observations from the actual values of state variables. The fitting procedure and its outcome depend on assumptions made about the relative contributions of process error and observation error to the total variance of the time series (Hilborn and Walters 1992).

We assumed that observation errors are much smaller than process errors. That assumption is reasonable because analyses of replicates yield standard deviations much smaller than the standard deviation among dates and because our enumeration procedures are based on counting subsamples until the standard error is less than 10% of the mean (Carpenter and Kitchell 1993; Lathrop and Carpenter 1992a). Under the assumption that observation error is negligible, the model fitting problem reduces to a regression of equation 3a (Draper and Smith 1981). These fits were performed in Matlab™ by minimizing the negative log likelihood using the simplex algorithm (MathWorks, Inc. 1990). Likelihoods were calculated using the normal distribution (Hilborn and Walters 1992). Parameter standard errors and correlations were calculated by the bootstrap procedure described by Hilborn and Walters (1992, p. 233–234).

Pairs of models were compared using Bayes' factors, or odds ratios (Reckhow 1990; Hilborn and Walters 1992; Jefferys and Berger 1992). Simply stated, the Bayes' factor is the odds given by the data that one model is better than another model. More precisely, the Bayes' factor represents the probability that one model is correct relative to the probability that another alternative model is correct, given a data set and that both models were equally probable prior to examination of the data (Berger and Delampady 1987). The Bayes' factor is the ratio of the likelihood of one model to that of another. Likelihoods calculated from the normal distribution were obtained from the curve fits. The larger the likelihood, the better the model fits. For example, a Bayes' factor of 100 means that the numerator model has a likelihood 100-fold greater than that of the denominator model. In terms of probabilities, the numerator model is 100 times more likely to be correct than the denominator model.

To test the assumption that observation error was negligible relative to process error, we performed stochastic simulations of equations 3a–3c. Model parameters and the process error standard deviation for equation 3a were obtained from the fit of a selected model (the base model). Time series were then simulated, adding observation errors through equations 3b and 3c. The standard deviation of the observation error was set equal to 20% of the standard deviation of the input series. This value is probably an upper bound on the actual observation error. One hundred such time series were simulated. The base ("true") model and an alternative model were then fit to each time series by the methods described above and compared via Bayes' factors. We recorded the number of times (out of 100 trial simulations) that the alternative ("wrong") model was selected as the best-fitting one. If adding observation error frequently led to selection of the wrong model, we inferred that our conclusions were sensitive to the assumption that observation error was negligible.

In certain comparisons, the model with the smaller residual error may not have the smaller prediction error (Walters 1986). This situation can arise when the model with the smaller residual has more parameters. In these cases, we compared the models using Akaike's information criterion (AIC), which adjusts the residual likelihood for the number of parameters in the model (Wei 1990).

Results

Predictions of the best-fitting models are compared with observations in Fig. 1. The models track some, but not all, of the fluctuations in the data. The standard deviation of residuals was 0.096 mg wet mass·L^{-1} in Paul Lake and 1.19 mg wet mass·L^{-1} in Lake Mendota. Relative to the normal distribution, residuals were leptokurtic. Most errors were small, but a few large errors occurred during blooms or crashes of algae. Autocorrelations

TABLE 2. Goodness of fit statistics for the eight models for time series of edible phytoplankton in Paul Lake ($n = 103$) and Lake Mendota ($n = 97$). Model number (see Table 1), number of parameters, residual standard deviation, and AIC are presented. Model number 0 is the simple alternative, $A(t + 1) = A(t)$, used as the denominator for the Bayes' factors in Fig. 2. Lower values of AIC indicate better-fitting models (Wei 1990).

Model	Number of parameters	Paul Lake		Lake Mendota	
		Residual s	AIC	Residual s	AIC
0	0	0.107	–406.4	1.705	103.51
1	2	0.112	–447.4	1.373	65.55
2	3	0.106	–456.1	1.194	40.41
3	3	0.105	–459.1	1.363	66.12
4	4	0.096	–475.6	1.192	42.10
5	1	0.109	–454.8	1.380	64.53
6	2	0.107	–456.8	1.713	108.47
7	3	0.105	–457.9	1.354	64.81
8	4	0.105	–455.9	1.366	68.44

were nonsignificant, except for lag 3 in Paul Lake ($r_3 = -0.242$). We did not attempt to account for this autocorrelation in the models.

Bayes' factors were used to compare the likelihood of the dynamic models with the likelihood of the previous value of the time series (i.e., the likelihood of the predictor $A(t + 1) = A(t)$). The previous value is a simple and logical predictor of the next value of the time series, and therefore a useful point of comparison for the models. In Fig. 2, base 10 logs of Bayes' factors are presented for the models versus the previous value of the time series. A log Bayes' factor of 2, for example, indicates that the model's likelihood is 10^2 times greater than that of the previous value of the time series. With respect to fitting future values of the time series, the odds on the model versus the previous value of the time series are 100:1. We assumed that an odds ratio of 10 or more (log Bayes' factor of 1 or more) indicated a notable difference in model performance.

In Paul Lake, model 4 (prey-dependent form with logistic algal growth and type II functional response) fit better than any other model (Fig. 2). Model 4 has a relatively large number of parameters. Nevertheless, it was also the best-fitting model on the basis of AIC (Table 2).

In Lake Mendota, models 4 and 2 (prey-dependent form with logistic algal growth and type I functional response) fit the data equally well and more closely than any other models (Fig. 2). With respect to AIC, model 2 is the best-fitting model (Table 2). This result indicates that the additional parameter of model 4 offers no advantage over model 2 and in fact increases the prediction error of model 4 relative to that of model 2.

Bayes' factors were also used to compare pairs of analogous models (Fig. 3). To compare algal growth terms, Bayes' factors were calculated for pairs of models that were identical except for algal growth term (models 2 and 1; 4 and 3; 6 and 5; 8 and 7). Likelihoods of logistic models were placed in the numerator, and likelihoods of linear models were placed in the denominator, so a positive log Bayes' factor indicates that the logistic model is superior. To compare grazing functional responses, the same procedure was followed for pairs of models that were identical except for the functional response (models 3 and 1; 4 and 2; 7 and 5; 8 and 6). Here the likelihood of the model with type II functional reponse was placed in the numerator, so a positive log Bayes' factor indicates that the type II model is superior. Prey-dependent and ratio-dependent forms were compared in the same way, using analogous pairs of models (models 1 and 5; 2 and 6; 3 and 7; 4 and 8). The likelihod of the prey-dependent form was placed in the numerator, so a positive log Bayes' factor indicates superiority of the prey-dependent form.

These data provide strong evidence for density-dependent (logistic) growth of edible phytoplankton (Fig. 3A and 3B). In Lake Mendota, models with logistic algal growth had likelihoods ~10^5 times larger than their counterparts with linear algal growth. In Paul Lake, models with logistic algal growth were superior for three model pairs out of four. Models 7 and 8 (ratio-dependent forms with type II functional responses) had equal likelihoods.

With respect to grazing functional responses, Bayes' factors favored the type II functional response in Paul Lake but were equivocal in Lake Mendota (Fig. 3C and 3D). In Paul Lake, the likelihood of the best-fitting model (4) was nearly 10^5 that of its analogue with type I functional response. In Lake Mendota, likelihoods were equal for three of the four model pairs and favored the type II functional response in the comparison of models 7 and 5 (ratio-dependent forms with linear algal growth). As noted above, AIC indicates that model 2, with a type I functional response, is the best model in Lake Mendota.

The comparisons of prey-dependent versus ratio-dependent equations favor prey dependence (Fig. 3E and 3F). The best-fitting models in both lakes are prey dependent. In Lake Mendota, prey-dependent forms fit better in three cases out of four, but the Bayes' factors were small. Log Bayes' factors of about 0.6 for the best-fitting models (2 and 4) indicate that their likelihoods are only about four times greater than the likelihoods of their ratio-dependent counterparts (models 6 and 8, respectively). In Paul Lake, the likelihood of the best-fitting model (4) was 10^4 times greater than the likelihood of its ratio-dependent counterpart. However, the other comparisons of prey-dependent and ratio-dependent models are equivocal (model 2 versus 6; 3 versus 7) or favor the ratio-dependent form (model 1 versus 5).

Our fitting procedure assumed that lack of fit to the models arose from process error, not observation error. Stochastic simulations tested for possible effects of observation error on the model comparisons (Table 3).

The superiority of the models with logistic algal growth is not an artifact of the assumption that process error is much larger than observation error (Table 3). Series were generated with model 3 (linear growth) and fits of model 3 were compared with fits of model 4 (logistic growth). Model 3 was correctly identified in 98% of the trials, despite the fact that it has fewer parameters than model 4. When linear growth is the underlying

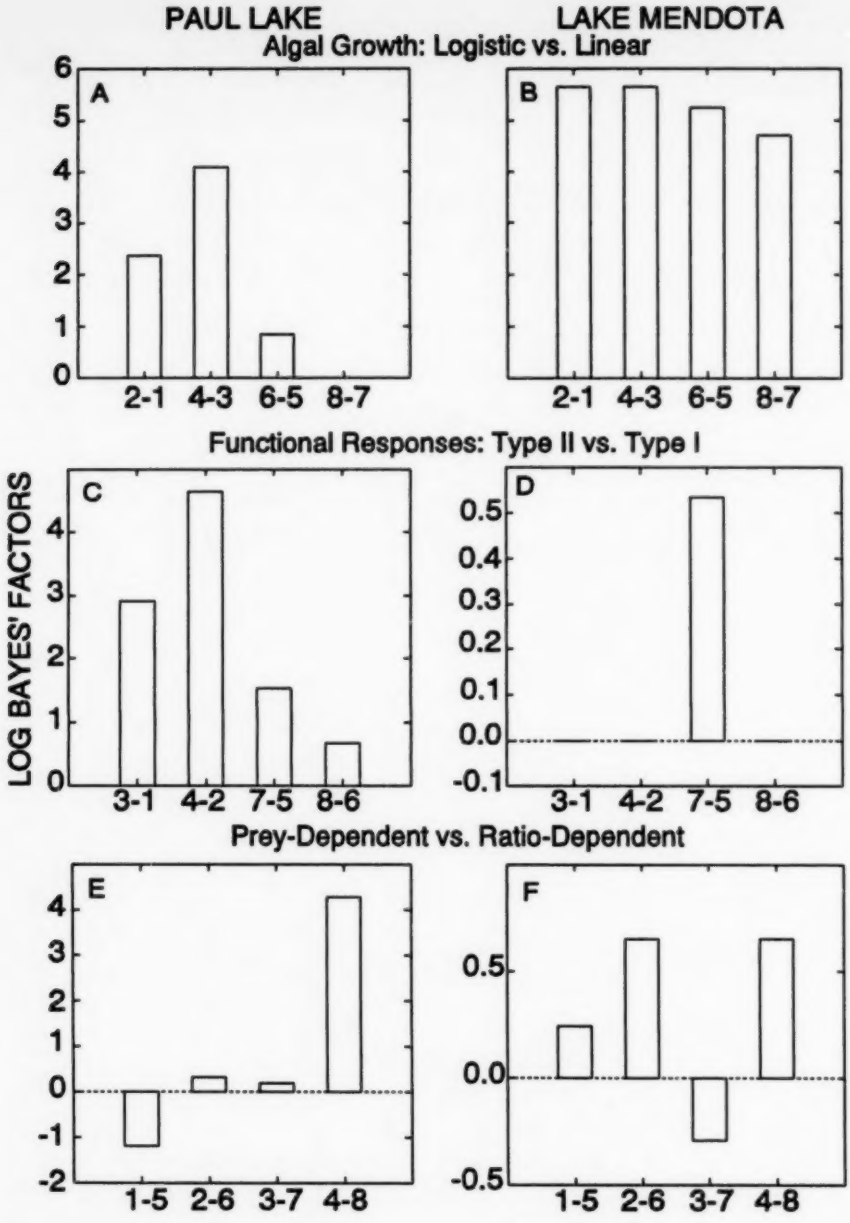


FIG. 3. Base 10 logarithm of Bayes' factor for comparisons of the models (Table 1). The top row compares models with logistic versus linear algal growth in (A) Paul Lake and (B) Lake Mendota; a positive log Bayes' factor indicates that the logistic model is superior. The middle row compares models with type II versus type I functional responses in (C) Paul Lake and (D) Lake Mendota; a positive log Bayes' factor indicates that the type II functional response is superior. The bottom row compares models with prey-dependent versus ratio-dependent forms in (E) Paul Lake and (F) Lake Mendota; a positive log Bayes' factor indicates that the prey-dependent formulation is superior.

model, we are not likely to misidentify logistic growth as the best-fitting model.

Comparisons of models with type I versus type II functional responses were more sensitive to observation error. Series were generated with model 2 (type I) and fits of model 2 were compared with fits of model 4 (type II). On the basis of Bayes' factors, the generating model was misidentified in about half of the simulations (Table 3). However, model 4 has more parameters than model 2. When AIC was used to adjust for the number of parameters, the correct model was identified in 100% of the

TABLE 3. Results of stochastic simulations to determine the effect of observation error on model comparisons. For each set of simulations, we report the model features that were contrasted, the lake that provided parameters for equation 3a, the base model (from Table 1) used to simulate the data using equations 3a–3c, the alternative model compared with the base model, error percentage (i.e., the percentage of trials that the alternative model fit better than the base model), and the 95% confidence interval for the percentage.

Model features contrasted	Lake	Base model	Alternative model	Error percentage	Confidence interval
Linear vs. logistic growth	Paul	3	4	2	0.2–7.2
Type II vs. type I functional response	Paul	2	4	51	40.8–61.1
Ratio-dependent vs prey-dependent functional response	Mendota	6	2	0	0–3.0
		7	3	42	32.3–52.3

TABLE 4. Parameter estimates and standard errors for models 2 and 4 in Paul Lake and Lake Mendota. Standard errors were obtained by linear regression for model 2 and by bootstrapping for model 4 (Hilborn and Walters 1992).

		Paul Lake		Lake Mendota	
Model	Parameter	Estimate	SE	Estimate	SE
2	c_1	0.062	0.018	0.00727	0.010
	c_2	0.202	0.052	0.00725	0.001
	c_3	0.045	0.023	0.00784	0.005
4	c_1	0.0624	0.011	0.00644	0.007
	c_2	0.202	0.040	0.00746	0.001
	c_3	441	8.6×10^{13}	13.9	8.7×10^{10}
	c_4	9750	2.9×10^{16}	1.19	2.0×10^{14}

simulations. Our conclusions about functional response based on AIC are not likely to be artifacts of the assumption that process error is larger than observation error.

Comparisons of models with ratio-dependent versus prey-dependent predation depended on whether the functional response was type I or type II. Using a type I functional response, series were generated with model 6 (ratio-dependent), and fits of model 6 were compared with fits of model 2 (prey-dependent). Model 6 was correctly identified in 100% of the simulations, even though model 2 has one more parameter. Under a type I functional response, if the generating model is ratio dependent, it is unlikely that the prey-dependent model will be misidentified as the best-fitting model. However, under a type II functional response, equivocal results are likely. Series were generated with model 7 (ratio dependent), and fits of model 7 were compared with fits of model 3 (prey dependent). The incorrect model was the best-fitting one in 42% of the simulations. Since models 7 and 3 have identical numbers of parameters, results are the same using AIC.

Parameter estimates for model 4 were confounded by near-perfect correlations of the estimates of the functional response parameters c_3 and c_4. Consequently, the standard deviations of these parameters are enormous (Table 4), and predictions made with these models will have very large errors. This is a major disadvantage of model 4, despite its relatively small error mean square in both lakes.

Parameter estimates for model 2 are much more satisfactory than those for model 4 (Table 4). The carrying capacity for edible algae, estimated as c_1/c_2, was 0.307 mg·L^{-1} in Paul Lake and 1.00 mg·L^{-1} in Lake Mendota. The lower carrying capacity of Paul Lake is consistent with its stained water and lower nutrient content (Carpenter and Kitchell 1993). Parameter standard errors are much smaller for model 2 than for model 4, and prediction errors using model 2 will therefore be much smaller than those using model 4.

Discussion

The correspondence of the best-fitting models to field data could be improved. The largest residuals are associated with unusually large peaks in algal biomass. These peaks are probably caused in part by nutrient pulses, mixing events, or algal recruitment events not included in the models. While the models we examined performed acceptably for average conditions, better predictions of algal blooms may require models that explicitly account for physical–chemical disturbances or recruitment of algae from benthic refugia.

This analysis indicates that growth of edible algae is negatively density dependent in both lakes. However, the mechanisms of density dependence may differ between the lakes, or even through time within a lake. Light extinction by humic substances probably contributes to the lower carrying capacity of Paul Lake (Elser 1987). Light limitation in Lake Mendota is more likely due to light extinction by algal chlorophyll (Carpenter 1992). In Lake Mendota, where high nutrients regularly but temporarily drive algal biomasses near or above carrying capacity, evidence for logistic algal growth is the strongest.

These data fit prey-dependent models better than ratio-dependent models. When the functional response is type I, prey dependence and ratio dependence can reliably be distinguished.

When the functional response is type II, observation error can make it very difficult to distinguish prey dependence from ratio dependence.

The choice of functional responses for grazing (type I versus type II) depends on whether the goal is to describe the variance in the data or to predict algal dynamics. With respect to fitting the data, the type II functional response is superior in Paul Lake. In Lake Mendota, the functional responses fit equally well. With respect to prediction, the type I functional response is superior in both lakes. The type II functional responses have severely correlated parameters and very large parameter variances. The best evidence for type II functional responses in plankton may be laboratory experiments such as those of Porter et al. (1982). It remains to be seen whether such experiments provide realistic parameter estimates at the ecosystem scale.

Discriminating nonlinear functional responses using ecological time series may prove difficult for a wide range of systems. Our stochastic simulations indicate that comparisons of these models are very sensitive to assumptions about the mixture of process versus observation error in the data. A similar problem in the analysis of fisheries stock–recruit data has been studied extensively (Ludwig and Walters 1981, 1989; Walters 1986; Hilborn and Walters 1992). Straightforward methods exist for fitting nonlinear models when errors can be assumed to be purely process or purely observational (Walters 1986; Hilborn and Walters 1992). When the data include both process and observation errors, as do some ecological time series, model fitting is more challenging. Most methods require an independent measure of observation error (Ludwig and Walters 1989). Hilborn and Walters (1992) recommended the approach taken here: fit the models under the assumption of either process error or observation error and then examine the consequences of the assumption using stochastic simulation. The outcome suggests that a method for fitting nonlinear functional responses to time series with both process and observation error is needed. Development of such a method is beyond the scope of this paper.

The best model for edible algae in these lakes has logistic growth and a prey-dependent, type I functional response (model 2). Model 4, which is identical to model 2 except for a type II functional response, fits about as well as model 2 in Lake Mendota and fits better than model 2 in Paul Lake. However, the parameters of the functional response in model 4 are strongly correlated and highly uncertain. This is a serious practical drawback to the use of model 4.

The best-fitting models for both lakes are prone to oscillations. In model 2, the edible algae – *Daphnia* system approaches equilibrium through damped oscillations. This also occurs in model 4 for certain combinations of parameter values. For other combinations of parameter values, model 4 yields a limit cycle (Rosenzweig 1971). Either model can explain the cycle from bloom to clear water observed in spring and early summer in many temperate lakes (Lampert et al. 1986; Carpenter 1992). Only part of a full cycle is seen in nature. The bloom of edible algae is stimulated by thawing of ice, nutrient pulses, wind mixing, and (or) warming of the water. Population growth of *Daphnia* follows the pulse of edible algae. Eventually the algae are overgrazed and *Daphnia* populations crash (Lampert et al. 1986; Carpenter 1992). With the onset of summer stratification, dominance shifts to inedible phytoplankton and smaller grazers, and there may be no opportunity for further cycles (Reynolds 1984; Sommer et al. 1986; Carpenter 1992). Therefore, it is impossible to tell whether the lake exhibits a damped oscillation or a limit cycle.

Regardless of the mechanism, we infer that the edible algae – *Daphnia* systems of these lakes display endogenous oscillations. This behavior is different from the four unproductive lakes studied by Walters et al. (1987). Plankton biomasses in their lakes were less than half of those found in Paul Lake and less than a tenth of those found in Lake Mendota. The trend from equilibrial behavior in nutrient-poor lakes to oscillatory behavior in nutrient-rich lakes is consistent with the paradox of enrichment (Rosenzweig 1971). However, we cannot demonstrate the limit cycles obtained in Rosenzweig's (1971) model. Because only part of a full cycle is seen in nature, this point may be irrelevant. A partial predator–prey cycle, perhaps initiated by early spring conditions far from equilibrium, is sufficient to produce the spring clear-water phase and *Daphnia* collapse that usher in the summer plankton community of more productive lakes (Carpenter 1992).

This study demonstrates an approach that links ecosystem models to ecological time series and thereby yields models that are firmly rooted in appropriately scaled data. Difficulties of extrapolating parameters from small-scale experiments are avoided. The approach also allows explicit comparison of multiple alternative hypotheses in the form of models. While our analysis is not unique in these respects, it is among only a handful of ecological and environmental studies that have attempted to discriminate alternative models using time series data (Reilly 1970; Blau and Neely 1975; Carpenter 1982; Walters 1986; Morris 1990; Hilborn and Walters 1992). In view of current efforts to develop long-term data bases in ecology (Likens 1989; Risser 1991), there should be increasing availability of data for analyses such as ours. Analyses of time series may significantly advance ecological modeling by identifying formulations that best fit field data, minimize forecast error, economize on numbers of parameters, and yield parameter estimates at the ecosystem scale.

Acknowledgments

We thank Kathy Cottingham, Tony Ives, Lars Rudstam, Anett Trebitz, and anonymous reviewers for helpful comments on the draft. The Andrew W. Mellon Foundation supports our research on modeling ecological time series. Data bases were developed under the support of the National Science Foundation, the Federal Aid to Sport Fish Restoration Act, and the Wisconsin Department of Natural Resources.

References

ARDITI, R., AND L.R. GINZBURG. 1989. Coupling in predator–prey dynamics: ratio-dependence. J. Theor. Biol. 139: 311–326.

BERGER, J.O., AND M. DELAMPADY. 1987. Testing precise hypotheses. Stat. Sci. 2: 317–352.

BERGQUIST, A.M., AND S.R. CARPENTER. 1986. Limnetic herbivory: effects on phytoplankton populations and primary production. Ecology 67: 1351–1360.

BLAU, G.E., AND W.B. NEELY. 1975. Mathematical model building with an application to determine the distribution of Dursban® insecticide added to a simulated ecosystem. Adv. Ecol. Res. 9: 133–163.

CARPENTER, S.R. 1982. Comparisons of equations for decay of particulate detritus in tree-hole ecosystems. Oikos 39: 17–23.

CARPENTER, S.R. 1992. Destabilization of planktonic ecosystems and blooms of blue-green algae, p. 461–482. *In* J.F. Kitchell [ed.] Food web management: a case study of Lake Mendota. Springer-Verlag, New York, N.Y.

CARPENTER, S.R., AND J.F. KITCHELL. 1987. The temporal scale of variance in limnetic primary productivity. Am. Nat. 129: 417–433.

CARPENTER, S.R., AND J.F. KITCHELL [ED.] 1993. The trophic cascade in lakes. Cambridge University Press, London, England.

DRAPER, N., AND H. SMITH. 1981. Applied regression analysis. John Wiley &

Sons., Inc., New York, N.Y. 709 p.
ELSER, J.J. 1987. Evaluation of size-related changes in chlorophyll-specific light extinction in some north temperate lakes. Arch. Hydrobiol. 111: 171–182.
ELSER, J.J., AND C.R. GOLDMAN. 1991. Zooplankton effects on phytoplankton in lakes of different trophic status. Limnol. Oceanogr. 36: 64–90.
FROST, T.M., D.L. DEANGELIS, S.M. BARTELL, D.J. HALL, AND S.H. HURLBERT. 1988. Scale in the design and interpretation of aquatic community research, p. 229–260. *In* S.R. Carpenter [ed.] Complex interactions in lake communities. Springer-Verlag, New York, N.Y.
HANSKI, I. 1991. The functional response of predators: worries about scale. Trends Ecol. Evol. 6: 141–142.
HASSELL, M.P. 1978. The dynamics of arthropod predator–prey systems. Princeton University Press, Princeton, N.J. 237 p.
HILBORN, R., AND C.J. WALTERS. 1992. Quantitative fisheries stock assessment. Chapman & Hall, London, England. 570 p.
JASSBY, A.D., T.M. POWELL, AND C.R. GOLDMAN. 1990. Interannual fluctuations in primary production: direct physical effects and the trophic cascade at Castle Lake, California. Limnol. Oceanogr. 35: 1021–1038.
JEFFERYS, W.H., AND J.O. BERGER. 1992. Ockham's razor and Bayesian analysis. Am. Sci. 80: 64–72.
KITCHELL, J.F. [ED.] 1992. Food web management: a case study of Lake Mendota. Springer-Verlag, New York, N.Y. 553 p.
LAMPERT, W., W. FLECKNER, H. RAI, AND B.E. TAYLOR. 1986. Phytoplankton control by grazing zooplankton: a study on the spring clear water phase. Limnol. Oceanogr. 31: 478–490.
LATHROP, R.C., AND S.R. CARPENTER. 1992a. Phytoplankton and their relationship to nutrients, p. 97–126. *In* J.F. Kitchell [ed.] Food web management: a case study of Lake Mendota. Springer-Verlag, New York, N.Y.
LATHROP, R.C., AND S.R. CARPENTER. 1992b. Zooplankton and their relationship to phytoplankton, p. 127–150. *In* J.F. Kitchell [ed.] Food web management: a case study of Lake Mendota. Springer-Verlag, New York, N.Y.
LIKENS, G.E. [ED.] 1989. Long-term studies in ecology. Springer-Verlag, New York, N.Y. 214 p.
LUDWIG, D., AND C.J. WALTERS. 1981. Measurement errors and uncertainty in parameter estimates for stock and recruitment. Can. J. Fish. Aquat. Sci. 38: 711–720.
LUDWIG, D., AND C.J. WALTERS. 1989. A robust method for parameter estimation from catch and effort data. Can. J. Fish. Aquat. Sci. 46: 137–144.
MATHWORKS, INC. 1990. Matlab™ for 80386-based MS-DOS personal computers. The MathWorks, Inc., Natick, Mass. 199 p.
MCCAULEY, E., AND W.W. MURDOCH. 1990. Predator–prey dynamics in environments rich and poor in nutrients. Nature (Lond.) 343: 455–457.
MORRIS, W.F. 1990. Problems in detecting chaotic behavior in natural populations by fitting simple discrete models. Ecology 71: 1849–1862.
MURDOCH, W.W., AND E. MCCAULEY. 1985. Three distinct types of dynamic behavior shown by a single planktonic system. Nature (Lond.) 316: 628–630.
PORTER, K.G., J. GERRITSEN, AND J.D. ORCUTT, JR. 1982. The effect of food concentration on swimming patterns, feeding behavior, ingestion, assimilation, and respiration by *Daphnia*. Limnol. Oceanogr. 27: 935–949.
RECKHOW, K.H. 1990. Bayesian inference in non-replicated ecological studies. Ecology 71: 2053–2059.
REILLY, P.M. 1970. Statistical methods in model discrimination. Can. J. Chem. Eng. 48: 168–173.
REYNOLDS, C.S. 1984. Phytoplankton periodicity: the interactions of form, function and environmental variability. Freshwater Biol. 14: 111–142.
RISSER, P.G. [ED.] 1991. Long-term ecological research: an international perspective. John Wiley & Sons, Inc., New York, N.Y. 294 p.
ROSENZWEIG, M.L. 1971. Paradox of enrichment: destabilization of exploitation ecosystems in ecological time. Science (Wash., D.C.) 171: 385–387.
RUDSTAM, L.G., R.C. LATHROP, AND S.R. CARPENTER. 1993. The rise and fall of a dominant planktivore: direct and indirect effects of zooplankton in Lake Mendota, Wisconsin. Ecology 74: 303–319.
SOMMER, U., Z.M. GLIWICZ, W. LAMPERT, AND A. DUNCAN. 1986. The PEG model of seasonal succession of planktonic events in fresh waters. Arch. Hydrobiol. 106: 433–471.
STEELE, J.H., AND E.W. HENDERSON. 1981. A simple plankton model. Am. Nat. 117: 676–691.
VANNI, M.J., S.R. CARPENTER, AND C. LUECKE. 1992. A simulation model of the interactions among nutrients, phytoplankton, and zooplankton in Lake Mendota, p. 427–450. *In* J.F. Kitchell [ed.] Food web management: a case study of Lake Mendota. Springer-Verlag, New York, N.Y.
WALTERS, C. 1986. Adaptive management of renewable resources. Macmillan Publishing Co., New York, N.Y. 374 p.
WALTERS, C.J., E. KRAUSE, W.E. NEILL, AND T.G. NORTHCOTE. 1987. Equilibrium models for seasonal dynamics of plankton biomass in four oligotrophic lakes. Can. J. Fish. Aquat. Sci. 44: 1002–1017.
WEI, W.W.S. 1990. Time series analysis. Addison-Wesley, New York, N.Y. 478 p.

Appendix

TABLE A1. Data used in the model fits. Symbols as in Table 1.

Lake Mendota				Paul Lake			
$A(t+1)$	$A(t)$	$Z(t)$	Δ_t	$A(t+1)$	$A(t)$	$Z(t)$	Δ_t
0.404	0.304	0.940	13	0.091	0.077	1.169	7
0.316	0.289	7.390	11	0.267	0.091	0.674	7
0.208	0.316	16.590	9	0.110	0.267	0.585	7
0.294	0.207	3.730	14	0.146	0.110	0.245	7
1.510	0.294	2.150	13	0.141	0.146	0.285	7
0.597	1.510	1.700	14	0.163	0.141	0.162	7
0.133	0.257	1.190	14	0.161	0.163	0.192	7
1.976	2.543	0.080	5	0.133	0.161	0.320	7
0.581	0.926	4.030	14	0.229	0.133	0.474	7
0.634	0.581	2.760	12	0.288	0.229	0.119	7
0.550	0.634	0.920	15	0.404	0.288	0.415	7
1.219	1.601	0.010	12	0.156	0.404	1.291	7
0.795	10.654	0.640	13	0.112	0.156	0.260	14
1.798	0.795	4.640	14	0.058	0.053	0.631	3
0.212	1.798	6.190	13	0.072	0.058	0.028	7
2.016	0.212	3.470	14	0.073	0.072	0.021	7
1.653	0.570	0.010	13	0.063	0.073	0.038	7
0.503	1.653	0.030	14	0.382	0.063	0.253	7
1.759	0.503	0.010	14	0.107	0.082	0.195	7
0.379	2.604	0.370	13	0.092	0.107	0.344	7
0.303	0.379	0.760	14	0.087	0.092	0.261	7
0.376	0.303	1.470	14	0.085	0.087	0.922	7
2.949	2.952	0.060	15	0.052	0.085	1.157	7
1.130	2.846	2.920	15	0.075	0.052	1.142	7
0.232	1.130	5.530	14	0.106	0.075	0.530	7

TABLE A1. *(Continued)*

Lake Mendota				Paul Lake			
$A(t+1)$	$A(t)$	$Z(t)$	Δ_t	$A(t+1)$	$A(t)$	$Z(t)$	Δ_t
0.239	0.232	13.020	15	0.057	0.106	0.469	7
0.941	0.239	0.090	13	0.057	0.057	0.489	7
1.345	0.941	0.590	15	0.041	0.057	0.403	7
3.591	2.301	0.050	14	0.067	0.041	0.876	7
0.384	3.591	0.580	14	0.078	0.067	0.341	14
0.155	0.384	6.200	15	0.048	0.035	0.229	7
0.065	0.155	7.570	14	0.117	0.048	0.206	7
0.558	0.065	3.570	13	0.175	0.117	0.148	7
1.254	3.872	0.210	14	0.203	0.175	0.213	7
0.089	1.254	1.720	14	0.073	0.203	0.459	7
0.058	0.089	9.060	14	0.107	0.073	0.362	7
0.189	0.058	1.280	14	0.094	0.107	0.093	7
0.039	0.189	0.120	14	0.157	0.094	0.093	7
1.967	3.375	0.120	14	0.206	0.157	0.033	7
3.325	1.967	0.030	15	0.207	0.206	0.147	7
7.972	3.325	0.250	11	0.189	0.207	0.115	7
0.014	0.011	5.760	13	0.200	0.189	0.271	7
0.119	0.014	3.850	13	0.231	0.200	0.081	7
0.162	0.119	1.980	14	0.104	0.231	0.113	7
0.832	0.162	1.570	15	0.081	0.076	0.430	7
2.493	0.832	0.380	14	0.068	0.081	0.883	7
0.041	2.493	0.010	14	0.058	0.068	0.848	7
1.257	1.652	0.170	14	0.058	0.058	0.384	7
4.994	1.257	0.020	14	0.116	0.058	0.095	7
1.937	4.994	0.170	14	0.143	0.116	0.098	7
0.046	1.937	1.670	14	0.073	0.143	0.040	7
0.184	0.046	7.270	13	0.079	0.073	0.259	7
0.691	0.184	2.910	14	0.485	0.079	0.022	7
0.719	0.691	0.240	15	0.317	0.485	0.078	7
0.444	0.719	0.130	13	0.297	0.317	0.372	7
2.602	2.028	0.070	15	0.155	0.297	0.412	7
2.354	2.602	0.030	14	0.215	0.155	0.574	7
0.167	2.354	0.470	14	0.182	0.215	0.607	7
0.395	0.167	10.800	14	0.359	0.182	1.227	7
0.086	0.395	12.570	14	0.261	0.189	0.122	7
0.164	0.086	4.210	14	0.174	0.261	0.152	7
0.892	0.164	0.560	14	0.159	0.174	0.079	7
0.622	0.892	0.710	14	0.122	0.159	0.098	7
0.463	0.622	0.250	14	0.268	0.122	0.235	7
0.278	0.463	0.020	15	0.323	0.268	0.492	7
2.152	2.115	0.100	12	0.251	0.323	0.181	7
1.795	2.152	0.130	7	0.176	0.251	0.626	7
6.811	1.795	0.130	6	0.094	0.176	0.364	7
1.718	6.811	0.020	7	0.176	0.094	0.548	7
3.790	4.609	0.530	6	0.170	0.176	0.226	7
4.916	3.790	1.040	8	0.569	0.170	0.405	7
0.431	4.916	1.470	6	0.436	0.569	0.234	7
0.047	0.431	4.960	8	0.418	0.436	0.261	7
0.116	0.047	11.140	7	0.118	0.418	0.913	6
0.322	0.116	4.860	6	0.069	0.148	0.035	7
0.187	0.322	2.830	7	0.167	0.069	0.178	7
0.179	0.187	1.230	7	0.385	0.167	0.179	7
0.308	0.179	2.580	7	0.132	0.385	0.815	7
0.723	0.308	0.160	7	0.070	0.132	0.120	7
0.412	0.723	0.250	7	0.207	0.070	0.159	7
0.472	0.266	0.160	8	0.059	0.207	0.008	7
0.400	0.472	1.010	7	0.120	0.059	0.203	7
0.406	0.400	0.530	6	0.067	0.120	0.269	7
0.249	0.406	0.270	8	0.134	0.067	0.160	7
0.209	0.249	1.700	6	0.146	0.134	0.037	7
0.286	0.209	0.130	15	0.230	0.146	0.248	7
0.249	0.286	0.140	14	0.537	0.230	0.248	7
0.234	0.339	0.020	11	0.110	0.114	0.218	7
0.218	0.234	1.620	14	0.027	0.110	0.114	7

TABLE A1. *(Concluded)*

Lake Mendota				Paul Lake			
$A(t+1)$	$A(t)$	$Z(t)$	Δ_t	$A(t+1)$	$A(t)$	$Z(t)$	Δ_t
0.067	0.218	1.740	14	0.044	0.027	0.386	7
0.242	0.067	1.450	14	0.058	0.044	0.025	7
0.053	0.242	2.000	14	0.020	0.058	0.824	7
0.124	0.124	0.780	15	0.078	0.020	0.041	7
0.293	0.124	0.790	14	0.192	0.078	0.121	7
0.895	0.730	0.530	14	0.170	0.192	0.098	7
0.047	0.895	2.250	14	0.142	0.170	0.050	7
0.404	0.118	0.370	14	0.078	0.142	0.094	7
—	—	—	—	0.150	0.078	0.766	7
—	—	—	—	0.077	0.150	0.606	7
—	—	—	—	0.088	0.077	0.324	7
—	—	—	—	0.048	0.088	0.331	6
—	—	—	—	0.255	0.048	0.619	9
—	—	—	—	0.337	0.255	0.400	6

Seasonal and Geographic Variation in the Species Composition and Size of Prey Consumed by Grey Seals (*Halichoerus grypus*) on the Scotian Shelf

W.D. Bowen, J.W. Lawson, and B. Beck

Department of Fisheries and Oceans, Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada

Bowen, W.D., J.W. Lawson, and B. Beck. 1993. Seasonal and geographic variation in the species composition and size of prey consumed by grey seals (*Halichoerus grypus*) on the Scotian Shelf. Can. J. Fish. Aquat. Sci. 50: 1768–1778.

The grey seal (*Halichoerus grypus*) population on the Scotian Shelf has grown significantly over the past 20 yr, thus increasing the potential for competitive interactions between grey seals and fisheries. The relative contribution (percent wet weight) and size of prey eaten were estimated from otoliths and squid beaks recovered from 143 stomachs that contained food of the 528 collected from 1988 to 1990. Although 22 taxa were found, only four species (Atlantic herring, silver hake, Atlantic cod, and squid) accounted for 80% of the estimated weight of food eaten. The mean length of prey eaten ranged from 19 to 35 cm for six species. Only 17% of the cod and none of the pollock and squid eaten were of the length taken in commercial fisheries. However, about 80% of the silver hake and herring eaten were of commercial size. Offshore at Sable Island, northern sand lance, silver hake, and squid (in order of importance) accounted for 86.1% of the wet weight ingested by seals during summer; sand lance and cod accounted for 96.1% of prey eaten in winter. At inshore locations, herring, cod, and pollock made up 90% of the diet in summer; Atlantic mackerel, cod, squid, and herring made up 83% of the diet in winter.

Les effectifs du phoque gris (*Halichoerus grypus*) de la plate-forme néo-écossaise ont considérablement augmenté au cours des vingt dernières années, ce qui a fait croître le risque de concurrence entre cette espèce et les pêcheurs. La contribution relative (pourcentage en poids frais) et la taille des proies dévorées ont été estimées à partir des otolithes et des becs de calmars retrouvés dans 143 estomacs sur les 528 examinés de 1988 à 1990. Même si on y a retrouvé 22 taxons, quatre espèces seulement (hareng de l'Atlantique, merlu argenté, morue franche et calmar) comptaient pour 80 % du poids estimatif de la nourriture ingérée. La longueur moyenne des proies dévorées variait de 19 à 35 cm chez six espèces. Seulement 17 % des morues et aucun des goberges ainsi que des calmars dévorés étaient de la longueur des prises commerciales. Toutefois, environ 80 % des merlus argentés et des harengs dévorés étaient de taille commerciale. Au large de l'île de Sable, le lançon, le merlu argenté et le calmar (dans l'ordre décroissant d'importance) comptaient pour 86,1 % du poids frais ingéré par les phoques au cours de l'été; le lançon et la morue comptaient pour 96,1 % des proies dévorées l'hiver. Dans les stations côtières, le hareng, la morue et la goberge constituaient 90 % du régime estival; le maquereau de l'Atlantique, la morue, le calmar et le hareng constituaient 83 % du régime hivernal.

Received September 8, 1992
Accepted March 5, 1993
(JB622)

Reçu le 8 septembre 1992
Accepté le 5 mars 1993

Pinnipeds are among the largest vertebrate piscivores in marine ecosystems. They are often abundant, particularly in polar and temperate waters. These characteristics have lead to the suggestion that pinnipeds may be significant consumers of fish and/or invertebrates and thus represent an important component of marine communities (Laws 1977). As potentially significant consumers of commercially important species, seals are often believed to negatively affect fisheries yields (e.g., Gulland 1987). However, despite their potential importance, quantitative estimates of the species composition of diets and size of prey eaten by pinnipeds are available for few species (e.g., George-Nascimento et al. 1985; Perez and Bigg 1986; Kapel and Angantyr 1989; Prime and Hammond 1990; Pierce et al. 1990).

To date, our knowledge of the diets of grey seals (*Halichoerus grypus*) in eastern Canada is based mainly on qualitative analyses of stomach contents (Fisher and MacKenzie 1955; Mansfield 1965; Mansfield and Beck 1977; Benoit and Bowen 1990a). These studies suggest considerable seasonal and geographic variation in diets using the relative frequency of occurrence as a measure of prey importance. Although the relative frequency of occurrence of prey has the advantage of being easy to compute, it provides little insight into the weight of different species consumed or the contribution of different species to the energy requirements of seals. Quantitative estimates of the percentage by weight of different species in the diet are limited to two summer samples and a late summer – early winter sample from the Gulf of St. Lawrence (Benoit and Bowen 1990b; Murie and Lavigne 1992). However, quantitative estimates of prey mortality caused by seals are needed if we are to understand the competitive interactions between seals and commercial fisheries (Lavigne et al. 1982; Harwood 1983; Beverton 1985).

In the northwest Atlantic, grey seals inhabit coastal and offshore waters from the Gulf of Maine to southern Labrador (Stobo et al. 1990). Although small breeding colonies occur on

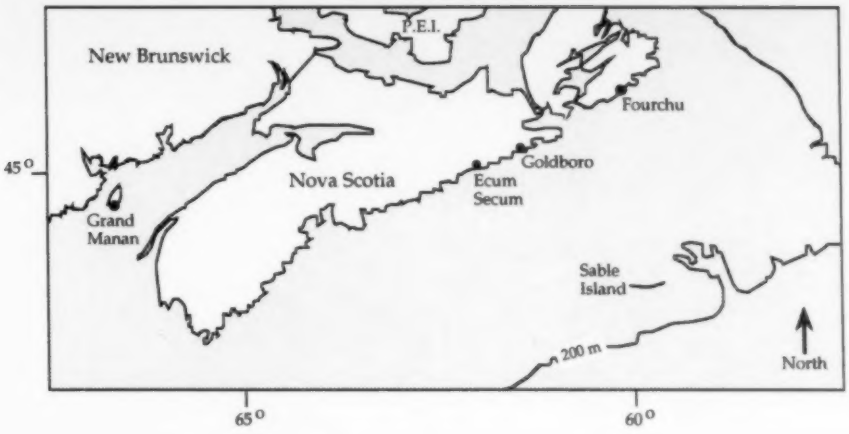


FIG. 1. Map showing the locations where grey seals were collected.

perhaps half a dozen coastal islands, over 90% of births take place on Sable Island and on the ice in the southern Gulf of St. Lawrence. Estimates of pup production in the Gulf of St. Lawrence and censuses conducted on Sable Island, on the Scotian Shelf, indicate that the number of grey seals has been increasing since the late 1960's (Zwanenburg and Bowen 1990). The total population of grey seals in eastern Canada in 1987 was estimated to be about 106 000 animals, but could have been as low as 84 000 or as high as 130 000 (Zwanenburg and Bowen 1990).

In their review of grey seal diets in eastern Canada, Benoit and Bowen (1990a) noted that there was significant geographic and seasonal variation in the frequency of occurrence of species consumed. They also noted that the lack of quantitative estimates of the percent wet weight of different species eaten and size of prey taken by seals limited the value of existing data in addressing questions about competitive interactions between grey seals and commercial fisheries. The main objects of this study were to estimate the seasonal and geographic variation in (1) the relative contribution, by wet weight and energy, of different prey species to the diets of grey seals and (2) the size of prey eaten by grey seals on the Scotian Shelf.

Materials and Methods

Stomachs were collected from 528 grey seals shot using high-powered rifles by three contracted hunters and one of the authors (B.B.) in three areas of the east coast of Canada: Grand Manan Island, New Brunswick; the eastern shore of Nova Scotia (including the towns of Ecum Secum, Forchu, and Goldboro), and Sable Island (Fig. 1). Samples were taken between September 1988 and November 1990. The Grand Manan and eastern shore samples were classified as "inshore" and the Sable Island samples were termed "offshore".

Seals were collected during most months of the year. However, there were too few samples to permit subdivision of the year either by month or according to events in the annual cycle of grey seals, such as breeding, moult, or dispersal. Thus, stomachs from seals collected between April and September were designated as "summer" samples and those taken between October and March as "winter" samples. Although the sex of adults could usually be determined before collection, the sex of juveniles could not. Therefore, we did not attempt to set up a balanced design with respect to sex. Given the difficulty in hunting grey seals, it was also not possible to obtain samples that accurately reflected the age structure. We used up to three age groups in the analysis: pups (<1 yr), age 1, and older than age 1. Ages were determined by counting annual growth layer groups in the cementum of a lower canine tooth as described by Hewer (1964).

In the field, each stomach was ligated at its oesophageal and pyloric sphincters and removed from the seal within 1–2 h of death. With the exception of 28 seals, the lower jaw was also removed and placed, along with the stomach, in a plastic bag with a tag noting the hunter's name, sex of the seal, location, and date of death. Stomachs were frozen within about 3 h of collection and maintained at about –20°C until analyzed.

Whole stomachs were thawed for approximately 15 h and then weighed on an electronic balance to the nearest 0.1 g. Each stomach was then opened in a large dissecting tray to prevent loss of contents. If present, whole prey items were removed and weighed to the nearest 0.1 g and measured to the nearest 1.0 mm (fork length in teleosts, or pen length in cephalopods). Saggital otoliths were removed from all whole fish heads recovered. Cephalopod beaks were removed from the buccal capsules of whole squids. Otoliths and squid beaks were stored in vials containing a preservative solution of 30% glycerine, 30% alcohol, and 40% water. The empty stomach was again weighed to determine the wet weight of the contents.

Free otoliths, squid beaks, and smaller invertebrate prey were recovered by the technique described by (Murie and Lavigne 1986). Previous work has demonstrated that over 90% of otoliths are recoverable using this method (Murie and Lavigne 1986; Benoit and Bowen 1990b). Recovered otoliths were examined under 16 or 40 power with a binocular dissecting microscope. Species were identified by comparing otoliths with reference material collected on the Scotian Shelf or with an otolith identification key (Härkönen 1986). In species where the left and right otoliths could be distinguished, the side with the greater number was used to determine the number of prey eaten. Where it was difficult to distinguish between left and right otoliths (e.g., those of northern sand lance or those that were partially eroded) the number of individuals consumed was estimated by dividing the total number of otoliths by two. Squid were identified by comparing either intact individuals, if present, or upper beaks

TABLE 1. Regression equations for calculating fork length (L), wet weight (W), and wet weight prey energy from body length, otolith, or beak measurements. Otolith length (OL) and width (OW) in mm.

Prey species	Regression equations for estimates of prey size and energy		
	Prey length (cm)	Wet weight (g)	Wet weight prey energy (kJ/g)
Northern sand lance	$L = -4.377 + 9.024(OL)$ [a]	$W = -0.371(OL)^{3.89}$ [a]	5.8 [b]
Atlantic herring	$L = 48.72(OL)^{1.35683}$ [c]	$W = 1.098(OL)^{4.24154}$ [c]	10.6 (summer), 7.9 (winter) [d]
Atlantic cod	$L = \exp\{1.8337 + 1.3536OL\}$ [e]	$W = 0.0084(L)^{3.02}$ [e]	4.8 [f]
Pollock	$L = 25.817(OL)\ 1.651$ [e]	$W = 0.015(L)^{2.93}$ [e]	5.0 [g]
Silver hake	$L = 20.288(OL)^{1.0239}$ [e]	$W = 0.014(L)^{2.92}$ [e]	6.0 [h]
Atlantic mackerel	$L = -2.041 + 8.759(OL)$ [c]	$W = 1.094(OL)^{4.039}$ [a]	9.9 [i]
American plaice	$L = 56.48(OL)^{1.2425}$ [e]	$W = 0.0042(L)^{3.197}$ [e]	5.1 [j]
Haddock	$L = \{\exp(2.0479 + 1.0087(OL))\}$ [e]	$W = 0.0077(L)^{3.073}$ [e]	
Winter flounder	$L = (W/A)^{-B}$ where $A = 0.0079$ and $B = 3.12$ [c]	$W = 0.0079(OL)^{3.12}$ [c]	
Yellowtail flounder	$L = 6.979 + 6.709(OL)$ [a]	$W = -161.05 + 57.78(OL)$ [a]	
Cusk	$L = (W/A)^{-B}$ where $A = 0.01$ and $B = 3.0$ [k]	$W = 0.01(OL)^{3.0}$ [k]	
Sea raven	$L = (W/A)^{-B}$ where $A = 0.0137$ and $B = 3.09$ [k]	$W = 0.0137(OL)^{3.09}$ [k]	
Squid	$L = 300.7(\log_{10}$ upper hood length$) - 123.92$ [l,m]	$W = 0.002(L)^{3.56}$ [n]	4.2 [i]

[a]This study.
[b]Summer and winter estimates from Hislop et al. (1991) for European fish.
[c]Estimate derived by applying a European regression from Härkönen (1986).
[d]Estimate derived by applying a European regression from Hislop et al. (1991).
[e]Benoit and Bowen (1990b).
[f]Holdway and Beamish (1984).
[g]Estimate derived from immature pollock taken in Norway by Jensen (1979).
[h]Kohler et al. (1970).
[i]Estimate derived using fat and protein proportions from Krzynowek and Murphy (1987).
[j]MacKinnon (1972).
[k]Joe Hunt, Department of Fisheries and Oceans, Marine Fish Division, St. Andrews Biological Station, St. Andrews, N.B., personal communication.
[l]Measured using the length of the upper hood of the beak from tip to base, L in mm.
[m]Lilly and Osborne (1984).
[n]Dawe (1984).

with published descriptions (Clarke 1986). The number of squid consumed was assumed to be equal to the number of the more numerous beak halves. Whole upper squid beaks were measured if their hoods were intact and showed no erosion of the beak tip or the posterior hood margin. Upper squid beaks were measured from the tip of the beak to the base of the hood (Mercer et al. 1980).

Only otoliths with minimal or no erosion were used to estimate the size of prey consumed. Degree of erosion was determined by comparing the surface and edge features of the recovered otolith with either those in our reference collection or Härkönen (1986). Otoliths that had complete surface detail and whose margins displayed a morphology similar to the reference material were measured to the nearest 0.1 mm using an image analysis system (Campana 1987). In most species, length measurements were taken from the rostrum to the posterior edge of the otolith, parallel to the sulcus. However, in butterfish, measurement was the greatest width of the otolith (Härkönen 1986). Length and wet weight of fish prey and squid were estimated from regressions relating otolith or beak dimensions to prey length and then using these lengths in length–weight regressions (Table 1). To estimate total biomass of prey in a stomach, we assumed that eroded otoliths of each species were originally the same size as the average of the uneroded measured otoliths. Estimated energy density (joules per gram wet weight) of prey was taken from the literature or derived using published estimates of the proportions of fat and protein and assuming that fat yields 39.3 J/g wet weight and protein yields 23.6 J/g wet weight (Blaxter 1989).

The scientific names of fish species referred to are given in Table 5. The standard error of the mean is used throughout.

Results

Proportion of Stomachs Containing Food

Although hunters collected samples in most months, few (27.1%) of the 528 grey seals examined in this study had prey remains in their stomachs. Furthermore, samples of stomachs that contained food were unevenly distributed among different areas and throughout the year (Table 2). However, there was considerable seasonal variation in the proportion of stomachs that contained food. Seals collected in summer were significantly ($\chi^2 = 41.2$, df = 1, $p < 0.001$) more likely to have had food in their stomachs (116 of 309 or 37.5%) than those collected in the winter (27 of 219 or 12.3%). Seals collected in August ($N = 87$) of each year had the highest percentage (69%) of stomachs that contained food. By contrast, none of the 63 stomachs collected from grey seals on Sable Island in March contained food. Geographic variation was also evident. Seals collected at inshore sites had a significantly higher ($\chi^2 = 49.0$, df = 1, $p < 0.001$) percentage of stomachs that contained food (41.4%) than those collected from Sable Island (13.6%). There was no significant difference between 261 males (24.1%) and 247 females (27.4%) in the percentage of stomachs that contained food ($\chi^2 = 0.596$, df = 1, $p = 0.44$). The percentage of stomachs that contained food also did not differ among 170 pups (25.9%), 185 yearlings (26.1%), and 145 older seals (26.7%) ($\chi^2 = 0.004$, p = 0.95).

TABLE 2. Locations, numbers, and monthly distribution of 143 grey seal stomachs containing food.

Year	Location	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1988	Grand Manan									4			
	Eastern Shore									14	3		
	Total									18	3		
1989	Sable Island		1			3	3		19	1	1		9
	Grand Manan	2					1						
	Eastern Shore							1	11	3			
	Total	2	1			3	4	1	30	4	1		9
1990	Grand Manan				3		2						
	Eastern Shore		1				5	4	30	12	3	7	
	Total		1		3		7	4	30	12	3	7	
	Overall total	2	2		3	3	11	5	60	34	7	7	9

TABLE 3. Comparison of otolith size and reconstructed prey size between loose and skull-recovered otoliths within the same nine stomachs that contained Atlantic herring and two stomachs that contained Atlantic cod.

			Otolith length (mm)		Prey length (cm)		Prey weight (g)	
		N	Mean	SE	Mean	SE	Mean	SE
Atlantic herring	Skull	32	5.1	0.07	37.9	0.65	644	41.8
	Loose	52	4.7	0.06	34.5	0.55	449	27.4
Atlantic cod	Skull	11	9.4	0.54	22.2	1.79	108	32.3
	Loose	6	9.5	0.62	24.1	2.20	161	53.0

Bias in Estimates of Prey Size

To investigate one source of bias in reconstructing prey length, we compared the lengths of otoliths removed from skulls and those found loose in the stomach and the derived measures of prey length and weight for Atlantic herring and Atlantic cod (Table 3). The lengths (nested ANOVA, $F = 2.75$, df = 17,66, $p = 0.002$) and weights (nested NOVA, $F = 3.09$, df = 17,66, $p = 0.001$) derived from loose herring otoliths were significantly less than those recovered from herring skulls found in the same nine stomachs. However, the estimated lengths ($F = 0.38$, df = 3,13, $p = 0.38$) and weights ($F = 0.92$, df = 3,13, $p = 0.46$) derived from cod otoliths found loose in two stomachs did not differ significantly from those recovered from skulls in the same stomachs.

The otoliths from silver hake, pollock, winter flounder, and yellowtail flounder were often badly eroded with the result that fewer than 25% of the recovered otoliths from these species were used to reconstruct prey size (Table 4). Many sand lance otoliths were also eroded but because of the large number of otoliths recovered, only 4% were measured.

Composition of Diet

Twenty-two different species and/or taxa were identified from the 143 grey seal stomachs that contained food (Table 5). However, five species (herring, cod, sand lance, silver hake, and squid) accounted for about 88% of the estimated weight of food eaten. Haddock was positively identified in only one stomach and consisted of a single individual. It is possible that haddock occurred among the otoliths representing 32 individuals that were identified as gadids only. Another 8 and 36 individuals could only be identified as unknown flatfish and clupeids, respectively, and a further 21 individuals were classified as unknown fish. Together these unknown individuals represented 3.5% of the total number of individuals recovered. Among the invertebrates identified, only squid appeared to have been eaten in significant quantities (Table 5). The small pieces of sand dollar, crab, and boreal shrimp found in grey seal stomachs likely represented secondary feeding, as these prey were always found in stomachs that also contained gadoid prey.

Seasonal and geographic variation in diets of grey seals was evident based on a comparison of the prey species that accounted for 5% or more of the overall (see Table 5) estimated wet weight of food consumed (Table 6). At inshore locations during summer, six species accounted for about 95% of the weight of food eaten, with herring and cod contributing most of the weight and energy ingested. During winter, five of these same species accounted for 84% of the estimated mass of food eaten. Atlantic mackerel provided almost 70% of the estimated energy intake, with herring, cod, and squid accounting for most of the remainder.

Offshore during summer, five species accounted for essentially all of the mass of food consumed by grey seals, with sand lance, silver hake, and squid being the most important species (Table 6). Only four species accounted for the mass of prey eaten during the winter period on Sable Island and two of these species, sand lance and cod, provided over 95% of the estimated energy ingested.

Effect of Seal Age on Diet Composition

Age was known for only 130 of the 143 seals whose stomachs contained food. To investigate whether pups had a different diet composition than older seals, we compared pups with the combined sample of fifty-eight 1-yr-olds and 28 older seals. Because pups and older seals were often not taken at the same location or in the same season, we did not feel that statistical analysis of these data was appropriate. There were few

TABLE 4. Prey type, number of otoliths recovered, percentage of otoliths measured, and estimated total number of individuals from otoliths and whole prey recovered in 143 grey seal stomachs containing food.

Prey type[a]	Total number of otoliths recovered	Total number of otoliths measured	Percent measured	Estimated number of individuals
Atlantic herring	347	160	46.1	204
Silver hake	223	43	26.0	138
White hake	26	11	42.3	16
Atlantic cod	341	115	33.7	187
Northern sand lance	3445	139	4.0	1724
Atlantic mackerel	2	1	50.0	8
American plaice	12	9	66.7	9
Haddock	2	0	0.0	1
Pollock	220	55	25	126
Winter flounder	10	2	20.0	5
Yellowtail flounder	117	14	12.0	65
Ocean pout	3	2	66.7	3
Cunner	4	2	50.0	3
Cusk	11	11	100.0	12
Sea raven	7	3	42.9	4
Butterfish	14	8	57.1	8
Squid (beaks)	222	74	33.3	154
Total[b]	4998	651	13.0	2667

[a]Scientific names given in Table 5.
[b]Not including unidentified otoliths.

TABLE 5. Prey recovered from the stomachs of 143 grey seals, all years and all areas combined.

Prey type	Relative freq. (%)	N^a (%)	Wet weight (kg) (%)
Atlantic herring (*Clupea harengus*)	12.6	204 (7.4)	96.7 (38.5)
Silver hake (*Merluccius bilinearis*)	6.3	138 (4.8)	22.1 (8.8)
White hake (*Urophycis tenuis*)	1.5	16 (0.6)	2.4 (1.0)
Atlantic cod (*Gadus morhua*)	12.6	187 (6.8)	48.1 (19.2)
Northern sand lance (*Ammodytes dubius*)	6.3	1724 (62.4)	32.3 (12.8)
Atlantic mackerel (*Scomber scombrus*)	0.7	8 (0.2)	6.7 (2.7)
American plaice (*Hippoglossoides platessoides*)	2.2	9 (0.3)	0.8 (0.3)
Haddock (*Melanogrammus aeglefinus*)	0.4	1 (0.04)	
Pollock (*Pollachius virens*)	10.8	126 (4.6)	14.0 (5.6)
Winter flounder (*Pleuronectes americanus*)	1.1	5 (0.2)	0.7 (0.3)
Yellowtail flounder (*Pleuronectes ferrigineus*)	2.6	65 (2.3)	4.7 (1.9)
Ocean pout (*Macrozoarces americanus*)	1.1	3 (0.1)	
Cunner (*Tautogolabrus adspersus*)	0.7	3 (0.1)	
Cusk (*Brosme brosme*)	1.1	12 (0.4)	1.0 (0.4)
Sea raven (*Hemitripterus americanus*)	0.7	4 (0.1)	0.3 (0.1)
Butterfish (*Peprilus triacanthus*)	0.7		
Skate (*Raja* sp.)	1.1	3 (0.1)	
Squid (*Illex illescebrosus*)	13.4	154 (5.6)	20.8 (8.3)
Gastropod	2.6	31 (1.1)	
Boreal shrimp (*Pandalus* sp.)		unk.	
Crab (*Cancer* sp.)	0.4	1 (0.03)	
Sand dollar		unk.	
Unknown gadoid Sp.	8.0	32 (1.1)	
Unknown clupeid Sp.	4.8	36 (1.3)	
Unknown flatfish Sp.	0.4	8 (0.3)	
Unknown fish Sp.	8.5	21 (0.8)	

[a]N = estimated total number of individuals of each prey species. unk. = unknown.

differences in the relative contributions of different prey species to the diets of pups and older seals (Table 7). Expressed in terms of percent wet weight, pups appeared to consume more silver hake and less squid than older seals. Cod, herring, sand lance, and silver hake accounted for 88 and 78% of the estimated weight of food eaten by pups and older seals, respectively.

Size of Prey Eaten

The estimated overall mean length and weight of prey eaten by grey seals were 25.0 ± 3.7 cm and 224 ± 10.6 g, respectively.

TABLE 6. Estimates of percent occurrence, wet weight (kg), and energy (MJ) derived from species representing 5% or more of estimated biomass eaten by grey seals (N = number of stomachs).

Prey type	Summer				Winter			
	% occurrence	% number	% wet weight	% energy	% occurrence	% number	% wet weight	% energy
		$N = 90$				$N = 16$		
Inshore								
Atlantic herring	18.8	35.3	65.8	82.4	4.5	6.5	9.0	10.3
Atlantic cod	14.8	21.0	17.6	10.0	9.1	19.6	13.9	9.6
Pollock	14.8	17.2	7.0	4.1				
Atlantic mackerel	0.6	0.2	0.4	0.4	4.5	15.2	47.4	67.5
Silver hake	4.5	4.4	3.2	2.3	4.5	2.2	0.7	0.6
Squid	7.4	3.7	1.4	0.7	27.3	23.9	12.9	7.9
Sample size	176	754	104.9	954.9	23	63	5.6	34.4
		$N = 26$				$N = 11$		
Offshore								
Silver hake	19.1	12.1	25.8	29.2	4.2	0.3	0.6	0.7
Atlantic cod	4.3	4.2	6.1	5.5	12.5	1.6	20.1	17.4
Pollock	4.3	3.3	6.8	6.3				
Northern sand lance	14.9	58.4	32.7	35.8	25.0	94.4	75.1	78.7
Squid	25.5	13.4	26.6	21.1	16.7	0.5	4.2	3.2
Sample size	45	784	21.1	111.5	25	1254	33.9	187.4

TABLE 7. Percent frequency of occurrence, number of individuals, and estimate of wet weight of prey in the diets of pups (N = 44) and juvenile/adult (N = 86) grey seals.

Prey type	Pups			Juveniles/adults		
	% frequency	N (%)	Wet weight (%)	% frequency	N (%)	Wet weight (%)
Northern sand lance	6.8	540 (65.1)	4.3 (7.0)	5.7	1184 (63.0)	10.6 (6.8)
Atlantic cod	17.0	63 (7.6)	15.3 (24.8)	10.6	111 (5.9)	29.3 (18.7)
Silver hake	5.7	56 (6.7)	10.7 (17.3)	8.5	70 (3.7)	11.3 (7.2)
Atlantic herring	10.2	57 (6.9)	23.8 (38.5)	15.6	140 (7.5)	68.4 (43.6)
Pollock	14.8	55 (6.6)	4.4 (7.1)	10.6	71 (3.8)	9.6 (6.1)
Squid	14.8	18 (2.2)	2.1 (3.3)	14.9	135 (7.2)	18.4 (11.7)
Other spp.	30.7	41 (4.9)	1.2 (1.9)	34.0	167 (8.9)	9.2 (5.9)
Total	88[a]	830[b]	61.8	160[a]	1878[b]	157.0

[a]Total number of prey occurrences in stomachs that contained food.
[b]Total number of individual prey in stomach that contained food.

TABLE 8. Estimated mean length and wet weights of prey items in grey seal stomachs for all years and areas combined. N is the number of individuals either measured as whole prey items or whose sizes were estimated from uneroded hard parts.

Prey type	N	Length (cm)		Wet weight (g)	
		Mean	SE	Mean	SE
Atlantic herring	160	34.5	0.47	486	21.4
Silver hake	43	25.4	1.03	213	29.9
White hake	12	22.2	1.48	135	25.2
Atlantic cod	115	25.8	0.92	231	26.4
Northern sand lance	139	19.1	0.31	17.5	0.8
Atlantic mackerel	8	20.9	5.05	710	171.4
American plaice	9	14.7	1.56	30	14.6
Pollock	55	20.0	1.04	139	20.6
Winter flounder	2	24.3	2.01	168	32.8
Yellowtail flounder	14	20.0	1.95	72	15.7
Cusk	11			94	20.9
Sea raven	3			97	57.0
Squid	78	18.5	0.32	136	6.2

TABLE 9. Estimated size of prey eaten by grey seals at inshore and offshore locations, all years.

			Length (cm)			Weight (g)		
	N	Area	Mean	SE	p^a	Mean	*SE*	p^a
Atlantic cod	95	Inshore	24.1	0.96		194	28.0	
	20	Offshore	33.7	1.81	0.0001	403	61.0	0.0023
Pollock	41	Inshore	20.2	1.18		141	24.1	
	14	Offshore	19.2	2.29	0.667	133	41.0	0.866
Squid	31	Inshore	17.3	0.51		114	8.9	
	47	Offshore	19.4	0.35	0.0007	150	7.8	0.0043

[a]Difference between areas.

TABLE 10. Size of selected prey from inshore sites during the period July–October 1989 and 1990.

		1989			1990			
	Species	*N*	Mean	SE	*N*	Mean	SE	*p*
Length (cm)	Atlantic herring	23	34.6	1.18	117	35.3	0.46	0.573
	Atlantic cod	17	20.2	0.99	38	21.2	1.05	0.550
	Pollock	9	14.6	2.3	23	19.2	1.04	0.046
Weight (g)	Atlantic herring	23	490	66.1	117	510	23.6	0.741
	Atlantic cod	17	83	13.7	38	112	18.9	0.336
	Pollock	9	62	22.0	23	103	21.1	0.269

[a]Difference between years.

Among the six species for which we had estimates from 30 or more individuals, the reconstructed mean length of prey varied from about 19 to 34 cm and the mean weight ranged from 18 to 486 g (Table 8). Based on recovered otoliths, the largest fish consumed by a seal in our sample was a cod 56.5 cm long weighing an estimated 1.6 kg.

The estimated mean length of prey eaten by grey seals from Sable Island (20.1 ± 3.71 cm, $N = 269$) was significantly less than that eaten at inshore sites (28.0 ± 5.21 cm, $n = 356$; $F = 113.5$, df = 1,623, $p < 0.001$), probably reflecting the greater number of sand lance consumed offshore. Only cod, pollock, and squid were frequently consumed at both offshore and inshore sites (Table 9). There were no significant differences in the estimated mean lengths or weights of pollock eaten at offshore and inshore locations. However, the mean length and weight of both cod and squid were significantly greater at the offshore location.

Three species occurred frequently enough in both 1989 and 1990 at the inshore locations along the eastern shore of Nova Scotia to examine interannual variability in the size of prey taken by grey seals (Table 10). We found no significant differences in the estimated mean length or weight of either herring or cod between the two years. However, the estimated mean length of pollock in the diet was significantly greater in 1990 than in 1989.

The estimated length–frequency distributions of six of the most commonly eaten species are given in Fig. 2. The cross-hatched bars represent the length of each species that would likely be taken by commercial fisheries, with the exception of sand lance, which are not commercially exploited. These data indicate that only about 17% of the cod eaten and none of the pollock and squid consumed by grey seals would have been taken by commercial fisheries. By contrast, most of the silver hake (80%) and herring (79%) found in the diet were of commercial size (Fig. 2).

Discussion

Although studies of grey seal diets in eastern Canada have spanned more than four decades (reviewed by Benoit and Bowen 1990a), with the exception of the recent studies by Benoit and Bowen (1990b) and Murie and Lavigne (1992) in the Gulf of St. Lawrence, the results of these studies have been expressed as the frequency of occurrence or the relative frequency of foods eaten. Thus, while the variety of foods eaten is well known, there is little information on the size or relative amount of different prey consumed by grey seals. Our data are the first quantitative estimates for the Scotian Shelf, an area where more than half of the grey seal pups in eastern Canada are born (Zwanenburg and Bowen 1990) and where a considerable fraction of the population is thought to feed.

Despite attempts to set up a balanced design by month and area for the collection of stomach samples, coverage varied (Table 2) such that seasonal and geographic effects on the diet are counfounded to some extent. This variation reflects not only the availability of seals near shore but also the effect of inclement weather on hunting effort and success rate. Thus, it seems unlikely that these difficulties can be eliminated. Further, only 72% of the overall sample of stomachs contained material that could be used to reconstruct the diet. The low percentage of stomachs that continued food was partly due to the fact that the timing of sample collection from Sable Island was dictated by other studies, such that samples were taken during the breeding season and moult period when few grey seals were feeding. These are features common to studies of seal diet using stomachs collected from animals that have been shot. They occur to a considerably lesser extent in faecal analysis, which has its own set of problems.

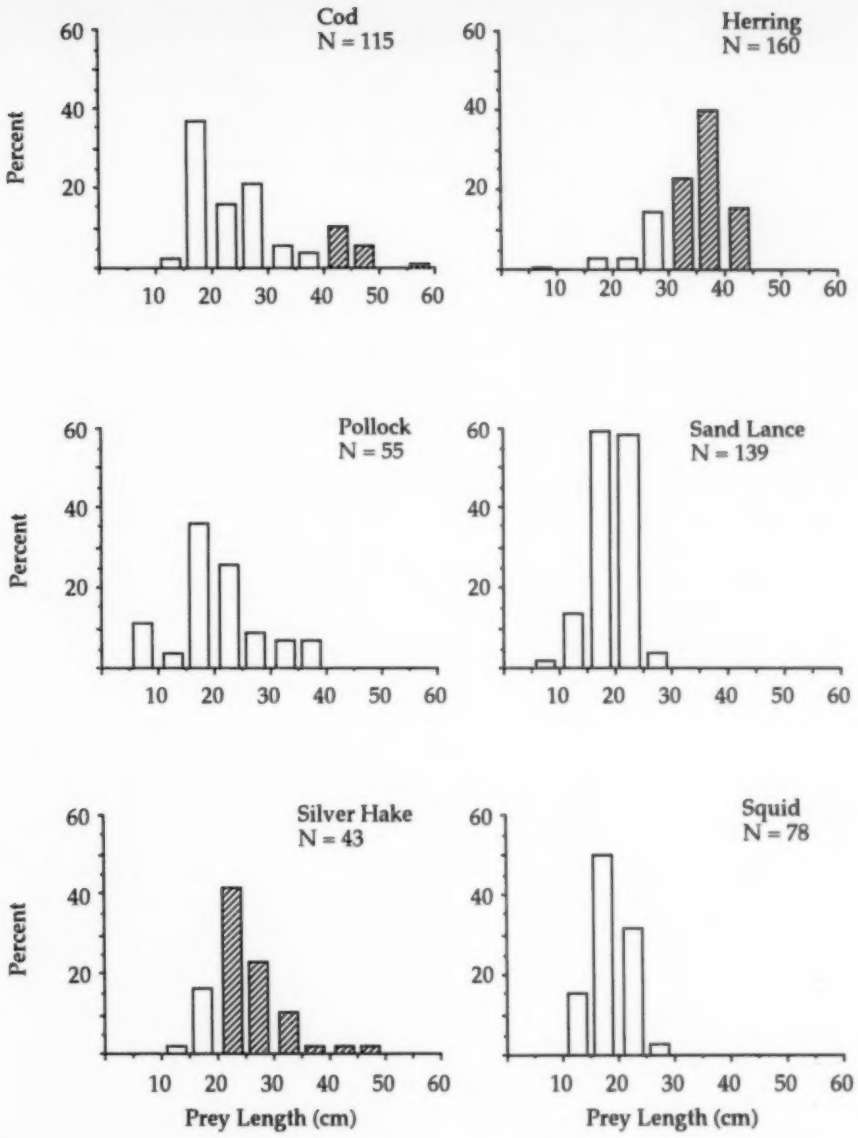


FIG 2. Distribution of estimated lengths of prey eaten by grey seals. Hatched bars indicate that prey length overlaps those in commercial fisheries. Note: commercial length for herring refers to the purse-seine fishery only; sand lance are not commercially exploited.

Otolith Erosion

Otoliths and other hard parts recovered from stomachs can be valuable in reconstructing the diet of seals, but their use is subject to various sources of bias (Jobling and Breiby 1986). Likely, the largest source of bias is the result of differences in the proportion of ingested otoliths of different species or of different-sized individuals of the same species that are significantly eroded or completely digested before the contents are examined (Jobling and Breiby 1986; Harvey 1989; Benoit and Bowen 1990b). In vitro studies have shown that herring otoliths are digested much more rapidly than the more robust otoliths of haddock (Jobling and Breiby 1986), suggesting that the relative importance of clupeids may be underestimated in reconstructed diets, while that of gadids may be overestimated. Murie and Lavigne (1985) found that 6 h after the ingestion of whole herring by captive grey seals, only about 70% of ingested otoliths could be recovered and that 46% of these recovered otolith showed evidence of erosion. Walleye pollock (*Theragra chalcogramma*) otoliths are robust and, like other gadid otoliths, are relatively resistant to digestion in ribbon seals (*Phoca fasciata*) (Frost and Lowry 1980) and northern fur seals (*Callorhinus ursinus*)

(Antonelis et al. 1986).

Recovered otoliths are often eroded to the point where species identification is uncertain. However, even when there is confidence in species identification, the use of eroded otoliths in diet reconstruction may significantly underestimate the size of prey eaten unless such erosion is accounted for (e.g., Prime and Hammond 1990). To reduce this source of bias, we only used otoliths that appeared to have retained their normal shape and edge topography. This approach may have reduced or eliminated the underestimation of the size of cod (see Table 3) and perhaps other gadids eaten by grey seals. However, our results suggest that loose otoliths may have underestimated the length and mass of herring eaten by 10 and 43%, respectively. By contrast, Benoit and Bowen (1990b) found no significant difference in the length of loose versus skull-recovered capelin (*Mallotus villosus*) otoliths in grey seal stomachs in the Gulf of St. Lawrence. Given that capelin also have fragile otoliths, it appears that factors other than otolith morphology can affect the degree of erosion.

Although we only measured "uneroded" otoliths, we made the assumption that eroded otoliths of the same species found in the same stomach came from the same size of fish as those we measured. If not true, this assumption would tend to bias the estimated prey size and thus the estimated weight and energy consumed. Unfortunately, the extent to which the various sources of bias offset one another cannot be determined at this time. Nonetheless, it is important to keep these potential sources of error in mind when interpreting the results of diet reconstruction.

Composition of the Diet

Based on the frequency of occurrence of prey in 207 stomachs that contained food collected from seven sites in eastern Canada, Mansfield and Beck (1977) concluded that grey seals relied on cod, flounders (*Pleuronectidae*), and skates (*Rajidae*) but took advantage of seasonally abundant pelagic species such as herring, mackerel, and squid. Our data confirm that only a few species (generally three to five) generally account for most of the food eaten by grey seals but suggest more dramatic seasonal and geographic changes in the species eaten than was indicated in previous studies (Mansfield and Beck 1977; Benoit and Bowen 1990a). In addition, our data identify sand lance, silver hake, and squid as seasonally important offshore foods of grey seals on the Scotia Shelf. Other studies have found a similar reliance by seals on a small number of prey species. Murie and Lavigne (1992) found that three species accounted for 72% of the estimated wet weight of prey consumed by grey seals in the northwest Gulf of St. Lawrence. Hammond and Prime (1990) also found that three to six species, including sandeels (*Ammodytes marinus*) and several gadids, accounted for 78–97% of the prey eaten by grey seals at several sites in the United Kingdom. Perez and Bigg (1986) found that three species typically accounted for 80% of the food eaten by the northern fur seal.

Our findings also indicate that there is substantial seasonal and geographic variation in the contribution of different prey species to the energy requirements of grey seals (Table 6). Although the overall pattern is similar to the estimated wet weight of prey consumed, differences in the energy content of prey both within and among species introduce additional variation. Expressed in terms of energy, cod and other species that have a low energy density contribute less to the diet per weight eaten than herring and other species of high energy density. The energy content of fish changes seasonally in relation to reproductive condition. Therefore, the relationship between the wet weight of food eaten and energy intake will vary. Furthermore, our data serve once again to illustrate, as others have pointed out (e.g., Lavigne et al. 1982; Benoit and Bowen 1990b; Murie and Lavigne 1992), that conclusions about the seasonal and geographic composition of diets based on frequency of occurrence data will often differ dramatically from those based on estimated wet weight or energy consumed.

Prey Size

Benoit and Bowen (1990b) reported that the mean length of fish eaten by grey seals appeared to be similar (i.e., about 25–35 cm) for a range of species despite differences in the adult length of these species. The exception was capelin (maximum adult length = 20 cm) where the mean length eaten by grey seals was about 14 cm. They concluded that grey seals fed primarily on schooling species such as herring, or on species, such as young cod, that are aggregated at this stage of their life history. Data from this study seem to confirm these previous findings. The mean length of prey eaten by grey seals on the Scotian Shelf varied between 19 cm (sand lance) and 34 cm (herring) with the mean lengths of three other species ranging between 20 and 26 cm. At Donna Nook in the southwestern North Sea, most of the sandeels and Dover sole (*Solea*) eaten by grey seals and roughly half of the cod were less than 35 cm in length (Prime and Hammond 1990). Bailey and Ainley (1982) reported that 60–98% of the Pacific hake (*Merluccius productus*) eaten by California sea lions were less than 41 cm in length. The mean length of walleye pollock eaten varied from 11 to 29 cm for four species of pinnipeds in the Bering Sea (Frost and Lowry 1986).

Within this range, the size of prey eaten will be affected by a number of factors including both the relative abundance of different-sized prey and the relative availability of prey. For example, during summer in the Gulf of St. Lawrence, Benoit and Bowen (1990b) found that the mean length of herring eaten by grey seals was 24.9 cm off Anticosti Island during summer compared with the mean of 34.5 cm from fall concentrations of herring off Cape Breton Island found in this study. Bailey and Ainley (1982) found that the proportion of Pacific hake in the diet of California sea lions appeared to track strong year-classes of hake, and thus the seals appeared to be responding to the relative abundance in the prey population. Our data indicate that both cod and squid taken at inshore locations were significantly smaller than those taken at the offshore site, Sable Island. The size of pollock consumed did not differ between the inshore and offshore locations but increased significantly from 1989 to 1990. There were no differences between years in the size of cod and herring eaten. There is also evidence that the size of prey consumed may vary with seal body size within and among pinniped species (Frost and Lowry 1986; Lowry et al. 1988).

During the summer near Anticosti Island in the Gulf of St. Lawrence, Benoit and Bowen (1990b) found little overlap between the length of fish eaten by grey seals and those taken in commercial fisheries. In the present study, less than 20% of the cod and none of the pollock or squid eaten by grey seals would have been taken in commerical fisheries. However, about 80% of the herring and silver hake found in the diet were of commercial size. As the herring data in our study and those in Benoit and Bowen (1990b) suggest, estimates of the fraction of prey of commercial size that are eaten by seals will vary at different times of the year and at different locations. Although more information is needed to make reliable estimates of

seasonal and/or geographic variation in the proportion of commercial-size prey in the diets of grey seals, current data suggest that the gadoids, such as cod and pollock, eaten by grey seals are for the most part small fish that are prerecruits to commercial fisheries.

Seasonal, geographic, and interannual variation in the species composition and relative importance of different prey in the diet of pinnipeds is well documented (Härkönen 1987; Kapel and Angantyr 1989; Benoit and Bowen 1990a, 1990b; Pierce et al. 1990; Hammond and Prime 1990; Prime and Hammond 1990; Pierce et al. 1991). Samples taken on Sable Island indicate that sand lance, silver hake, and squid are most important during the summer, but sand lance and young cod dominate the diet during the winter. At inshore sites, herring are apparently replaced by mackerel during the early winter as the most important food, with young cod again appearing in the diet throughout the year. Given the dynamic nature of most of the prey populations upon which seals feed, this variation is not unexpected. However, it does mean that predicting the composition of seal diets will be difficult without a better understanding of the availability of prey to seals. Availability will depend on the relative abundance and distribution of different prey species or size groups within species. If grey seal diets track strong year-classes within prey populations, then predicting the diet of seals may be tantamount to predicting the relative year-class size of the major prey species. Clearly, a sample of 143 stomachs that contain food is insufficient to characterize adequately the factors that affect the composition of grey seals diets over time. Long-term studies that are integrated with fisheries ecology programs are needed to provide the basis for predicting the composition of seal diets and for estimating the fish mortality caused by grey seals.

Acknowledgements

We wish to thank J. Hunt at St. Andrews Biological Station, St. Andrews, New Brunswick, for identifying flatfish otoliths. G. Forbes, Officer-in-Charge, of the Atmospheric Environmental Service, Environment Canada, greatly assisted our work on Sable Island and we thank him for his interest and support of our research. We also thank W.T. Stobo and G. Harrison for reviewing an earlier draft of the manuscript and two referees for their helpful suggestions.

References

ANTONELIS, G.A., JR., P. GEARIN, R.L. DELONG, AND T.R. LOUGHLIN. 1986. Fur seal food habits identified from scats and colons, p. 23–30. In summary of joint research on the diets of northern fur seals and fish in the Bering Sea during 1985. NWAFC Processed Report 86-19.

BAILEY, K.M., AND D.G. AINLEY. 1982. The dynamics of California sea lion predation on Pacific hake. Fish. Res. 1: 163–176.

BENOIT, D., AND W.D. BOWEN. 1990a. Seasonal and geographic variation in the diet of Grey seal (*Halichoerus grypus*) in eastern Canada, p. 215–226. *In* W.D. Bowen [ed.] Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and seal hosts. Can. Bull. Fish. Aquat. Sci. 222.

BENOIT, D., AND W.D. BOWEN. 1990b. Summer diet of Grey seal (*Halichoerus grypus*) at Anticosti Island, Gulf of St. Lawrence, Canada, p. 227–242. *In* W.D. Bowen [ed.] Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and seal hosts. Can. Bull. Fish. Aquat. Sci. 222.

BEVERTON, R.J.H. 1985. Analysis of marine mammal – fisheries interaction, p. 3–33. *In* J.R. Beddington, R.J.H. Beverton, and D.M. Lavigne [ed.] Marine mammal and fisheries. George Allen & Unwin (Publishers), London.

BLAXTER, K. 1989. Energy metabolism in animals and man. Cambridge University Press, Cambridge.

CAMPANA, S.E. 1987. Image analysis for microscope-based observations: an inexpensive configuration. Can. Tech. Rep. Fish. Aquat. Sci. 1569: 1–20.

CLARKE, M.R. [ed.] 1986. A handbook for the identification of cephalopod beaks. Claredon Press, Oxford.

DAWE, E.G. 1984. Variation in length–weight relationships, condition, and feeding spectrum of short-finned squid at Holyrood, Newfoundland. Northwest Atlantic Fisheries Organization SCR Document, Dartmouth, N.S.

FISHER, H.D., and B.A.A. Mackenzie. 1955. Food habits of seals in the Maritimes. Fish. Res. Board Can. Prog. Rep. 61: 5–9.

FROST, K.J., AND L.F. LOWRY. 1980. Feeding of ribbon seal (*Phoca fasciata*) in the Bering Sea in spring. Can. J. Zool. 58: 1601–1607.

FROST, K.J., AND L.F. LOWRY. 1986. Sizes of walleye pollock, *Thergra chalcogramma*, consumed by marine mammals in the Bering Sea. Fish. Bull. 84: 192–197.

GEORGE-NASCIMENTO, M., R. BUSTAMANTE, AND C. OYARZUN. 1985. Feeding ecology of the South American sea lion *Otario flavescens*: food contents and food selectivity. Mar. Ecol. Prog. Ser. 21: 135–143.

GULLAND, J.A. 1987. The impact of seals of fisheries. Mar. Policy 11: 196–204.

HAMMOND, P.S., AND J.H. PRIME. 1990. The diet of British grey seals (*Halichoreus grypus*), p. 243–254. *In* W.D. Bowen [ed.] Population biology of sealworm (*Psuedoterranova decipiens*) in relation to its intermediate and seal hosts. Can. Bull. Fish. Aquat. Sci. 222.

HÄRKÖNEN, T., 1986. Guide to the otoliths of the bony fishes of the Northeast Atlantic. Danbiu ApS., Biological Consultants, Hellerup, Denmark.

HÄRKÖNEN, T.J. 1987. Seasonal and regional variations in the feeding habits of the harbour seal, *Phoca vitulina*, in the Skagerrak and the Kattegat. J. Zool. (Lond.) 213: 535–543.

HARVEY, J.T. 1989. Assessment of errors associated with harbour seal (*Phoca vitulina*) faecal sampling. J. Zool. (Lond.) 219: 101–111.

HARWOOD, J. 1983. Interactions between marine mammals and fisheries. Adv. Appl. Biol. p. 189–214.

HEWER, H.R. 1964. The determination of age, sexual maturity, longevity and a life-table in the grey seal (*Halichoerus grypus*). Proc. Zool. Soc. Lond. 142: 593–634.

HISLOP, J.R.G., M.P. HARRIS, AND J.G.M. SMITH. 1991. Variation in the caloric value and total energy content of the lesser sandeel (*Ammodytes marinus*) and other fish preyed on by seabirds. J. Zool. (Lond.) 224: 501–517.

HOLDWAY, D.A., AND F.W.H. BEAMISH. 1984. Specific growth rate and proximate composition of Atlantic cod (*Gadus morhua* L.). J. Exp. Mar. Biol. Ecol. 81: 147–170.

JENSEN, A.R. 1979. Energy content analysis from weight and liver index measurements of immature pollock (*Pollachius virens*). J. Fish. Res. Board Can. 36: 1207–1213.

JOBLING, M., AND A. BREIBY. 1986. The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. Sarsia 71: 265–274.

KAPEL, F.O., AND L.A. ANGANTYR. 1989. Feeding patterns of harp seals (*Phoca groenlandica*) in coastal waters of west Greenland, with a note on offshore feeding. International Council for the Exploration of the Sea, Marine Mammal Committee C.M. 1989/N:6, Copenhagen, Denmark.

KOHLER, A.C., D.N. FITZGERALD, R.G. HALLIDAY, J.S. SCOTT, AND A.V. TYLER. 1970. Length–weight relationships of marine fishes of the Canadian Atlantic region. Fish. Res. Board Can. Tech. Rep. 164.

KRZYNOWEK, J., AND J. MURPHY. 1987. Proximate composition, energy, fatty acid, sodium, and cholesterol content of finfish, shellfish, and their products. NOAA Tech. Rep. NMFS 55: 53 p.

LAVIGNE, D.M., W.W. BARCHARD, S. INNES, AND N.A. ORITSLAND. 1982. Pinniped bioenergetics, p. 191–235. *In* Mammals in the seas, small cetaceans, seals, sirenians and otters. Food and Agriculture Organization of the United Nations, Fish. Ser. No. 5., Rome.

LAWS, R.M. 1977. The significance of vertebrates in the Antarctic marine ecosystem, p. 411–438. *In* G.A. Llano [ed.] Adaptation within Antarctic ecosystems. Symposium on Antarctic Biology. 3rd. Smithsonian Institution. Washington, D.C.

LILLY, G.R., AND D.R. OSBORNE. 1984. Predation by Atlantic cod (*Gadus morhua*) on short-finned squid (*Illex illecebrosus*) off eastern Newfoundland and in the northeastern Gulf of St. Lawrence. NAFO SCR Doc. 84/X1/108, Ser. N905.

LOWRY, L.F., K.J. FROST, AND T.R. LOUGHLIN. 1988. Importance of walleye pollock in the diets of marine mammals in the Gulf of Alaska and Bering Sea, and implications for fishery management. Proc. Int. Symp. Biol. Manage. Walleye Pollock, Anchorage, Alaska.

MACKINNON, J.C. 1972. Summer storage of energy and its use for winter metabolism and gonad maturation in American plaice (*Hippoglossoides platessoides*). J. Fish. Res. Board Can. 29: 1749–1759.

MANSFIELD, A.W. 1965. The grey seal, *Halichoerus grypus* (Fabricius), in eastern Canadian waters. Fish. Res. Board Can. MS Rep. (Biol. Ser.) 846: 1–25.

MANSFIELD, A.W., AND B. BECK. 1977. The grey seal in eastern Canada. Fish. Mar. Serv. Tech. Rep. 704.

MERCER, M.C., R.K. MISRA, AND G.V. HURLEY. 1980. Sex determination of the ommastrephid squid *Illex illecebrosus* using beak morphometrics. Can. J. Fish. Aquat. Sci. 37: 283–286.

MURIE, D.J., AND D.M. LAVIGNE. 1985. Digestion and retention of Atlantic herring otoliths in the stomachs of grey seals, p. 292–299. *In* J.R. Beddington, R.J.H. Beverton, and D.M. Lavigne [ed.] Marine mammal and fisheries. George Allen & Unwin (Publishers), London.

MURIE, D.J., AND D.M. LAVIGNE. 1986. Interpretation of otoliths in stomach content analysis of phocid seals: quantifying fish consumption. Can. J. Zool. 64: 1152–1157.

MURIE, D.J., AND D.M. LAVIGNE. 1992. Growth and feeding habits of grey seals (*Halichoerus grypus*) in the northwestern Gulf of St. Lawrence, Canada. Can. J. Zool. 70: 1604–1613.

PEREZ, M.A., AND M.A. BIGG. 1986. Diet of northern fur seals, *Callorhinus ursinus*, off western North America. Fish. Bull. 84: 957–971.

PIERCE, G.J., P.R. BOYLE, AND J.S.W. DIACK. 1991. Digestive tract contents of seals in Scottish waters: comparison of samples from salmon nets elsewhere. J. Zool. (Lond.) 225: 670–676.

PIERCE, G.J., P.R. BOYLE, AND P.M. THOMPSON. 1990. Diet selection by seals, p. 222–238. *In* M. Barnes and R.N. Gibson [ed.] Trophic relationships in the marine environment: Proceedings of the 24th European Marine Biology Symposium. Aberdeen University Press, Aberdeen, Scotland.

PRIME, J.H., AND P.S. HAMMOND. 1990. The diet of grey seals from the south-western North Sea assessed from analyses of hard parts found in faeces. J. Appl. Ecol. 27: 435–447.

STOBO, W.T., B. BECK, AND J.K. HORNE. 1990. Seasonal movements of grey seals (*Halichoerus grypus*) in the Northwest Atlantic, p. 199–213. *In* W.D. Bowen [ed.] Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and seal hosts. Can. Bull. Fish. Aquat. Sci. 222.

ZWANENBURG, K.C.T., AND W.D. BOWEN. 1990. Population trends of the grey seal (*Halichoerus grypus*) in eastern Canada, p. 185–197. *In* W.D. Bowen [ed.] Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and seal hosts. Can. Bull. Fish. Aquat. Sci. 222.

Application of Near-Infrared Reflectance Spectroscopy in the Measurement of Carbon, Nitrogen, and Phosphorus in Seston from Oligotrophic Lakes

D.F. Malley

Department of Fisheries and Oceans, Freshwater Institute, Central and Arctic Region, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada

P.C. Williams

Grain Research Laboratory Division, Canadian Grain Commission, 1401-303 Main Street, Winnipeg, MB R3C 3G8, Canada

and M.P. Stainton and B.W. Hauser

Department of Fisheries and Oceans, Freshwater Institute, Central and Arctic Region, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada

Malley, D.F., P.C. Williams, M.P. Stainton, and B.W. Hauser. 1993. Application of near-infrared reflectance spectroscopy in the measurement of carbon, nitrogen, and phosphorus in seston from oligotrophic lakes. Can. J. Fish. Aquat. Sci. 50: 1779–1785.

The application of near-infrared spectroscopy (NIRS), widely used in the agricultural, food, and pharmaceutical industries, is extended to the measurement of C, N, and P in seston from oligotrophic lakes at the Experimental Lakes Area, northwestern Ontario. Seston from 150-mL aliquots of lake water from several depths in two lakes on one mid-June sampling date was collected on Whatman GF/C filters and measured first by NIRS then analyzed by standard wet chemistry methods. Correlation between chemically measured and NIRS-predicted values gave coefficients of determination between 0.913 and 0.982. The rapid, low-cost, nonchemical, nondestructive, efficient analysis of C, N, and P in seston by NIRS is feasible.

La spectroscopie dans le proche infrarouge, largement utilisée dans les industries agroalimentaire et pharmaceutique, trouve une application élargie dans le dosage de C, de N et de P du seston des lacs oligotrophes de la région des lacs expérimentaux du nord-ouest de l'Ontario. Le seston renfermé dans des volumes de 150 mL d'eau lacustre prélevée à plusieurs profondeurs, dans deux lacs, à la même date (à la mi-juin), a été retenu sur des filtres Whatman GF/C et mesuré d'abord par spectroscopie dans le proche infrarouge, puis analysé au moyen des méthodes classiques de dosage en milieu aqueux. La corrélation entre les valeurs mesurées par voie chimique et prédites par la spectroscopie dans le proche infrarouge a donné des coefficients de détermination entre 0,913 et 0,982. Il est possible de doser C, N et P du seston, par la méthode rapide, peu coûteuse, efficace, non destructive et non chimique de spectroscopie dans le proche infrarouge.

Received October 26, 1992
Accepted March 11, 1993
(JB671)

Reçu le 26 octobre 1992
Accepté le 11 mars 1993

The measurement of particulate C, N, and P in freshwaters is essential to an understanding of C, N, and P cycles (Sandoval et al. 1976) and of the partitioning of particulate-bound materials, such as organic contaminants and heavy metals (Eisenreich and Hollod 1979; Bierman and Swain 1982; Lum 1987).

Particles are defined and collected by filtration or centrifugation, with Whatman GF/C filters being a commonly used membrane. Current methods for measuring particulate C and N usually involve either high-temperature combustion with measurement of CO_2 and N_2 or chemical digestion with measurement of CO_2 and NH_4. Methods for particulate P involve chemical digestion and measurement of PO_4. In all cases the analysis is destructive, time consuming, and relatively costly. Information yielded is an operationally defined approximation of total element content. Methods respond nonselectively to C, N, and P of both inorganic and organic origin.

Near-infrared reflectance spectroscopy (NIRS) is a rapid (45 s), chemical-free, nondestructive technique that has been used for the determination of many constituents and properties in the agriculture, food, textile, pharmaceutical, paper, and petrochemical industries. NIRS is a marriage between applied spectroscopy and statistics. Chemical bonds between light atoms such as C, N, and P generally have high vibrational frequencies and strong vibrational overtones detectable in the near-infrared region. The NIRS instruments detect the absorbances in the near-infrared region (300–2500 nm) caused by overtones and combination bands arising from fundamental absorbances in the midinfrared region (mainly 3000–15 000 nm). The absorbances are amplified, digitized, and computed into composition data, using equations developed during the calibration procedure. A linear relationship between absorbance and concentration, i.e., the Beer/Lambert/Bouguer relationship, is exhibited in the majority of biological and agricultural applications. The theory is discussed in Osborne and Fearn (1986), Williams and Norris (1987), and Williams (1991). This technique has been proclaimed by Wetzel (1983) as a "sleeper" among analytical techniques.

Since 1973, nearly 3000 scientific publications have appeared on the theory and applications of NIRS (F.E. Barton III, President, Council for Near-infrared Reflectance, USA, 1992,

personal communication). Of these, only one (Mathias et al. 1987) concerns aquatic biology where the technique was introduced for the analysis of lipid and protein contents of freshwater fish.

The present paper is the first attempt to apply NIRS to the field of aquatic chemistry. We assess the applicability of NIRS in determining concentrations of particulate C, N and P in the water column from two Precambrian Shield lakes by comparing NIRS-predicted values with those determined by the standard wet chemical methods.

Materials and Methods

Study Lakes

Water was sampled from two lakes, Lake 239 (49°39′N, 93°43′W, surface area 56.1 ha, maximum depth 30.4 m) and Lake 382 (49°42′N, 93°41′W, surface area 36.9 ha, maximum depth 13.1 m), in the Experimental Lakes Area (ELA), northwestern Ontario, described by Brunskill and Schindler (1971).

Both lakes are representative of the oligotrophic, softwater lakes in the ELA whose water chemistry is described in Armstrong and Schindler (1971) and Prokopowich (1979). A summary of the chemistry of the epilimnia of Lakes 239 and 382 during 1991 is given in Table 1. Beginning in June 1987, Lake 382 has been subject to experimental additions of Cd to describe the fate and effects of low levels of Cd contamination (Malley et al. 1989, 1991). The Cd additions to the lake have not had measurable impact on the water chemistry (unpublished data) or algal productivity (E. Schindler, Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man., personal communication). Although Lake 239 has not been experimentally manipulated, it has been impacted by forest fires and the presence of a research camp.

TABLE 1. Chemical composition (mean ± SE; pH = range) of the epilimnetic water of Lakes 239 and 382 during the ice-free season of 1991.

Parameter	Lake 239[a]	Lake 382[b]
Conductivity, µS/cm at 25°C	35.46 ± 0.24	24.42 ± 0.14
Dissolved inorganic carbon, µmol/L	186.0 ± 5.1	136.67 ± 4.61
pH, range	6.68–7.39	6.92–7.16
Calcium, mg/L	3.37 ± 0.11	2.44 ± 0.03
Magnesium, mg/L	1.03 ± 0.01	0.69 ± 0.01
Sodium, mg/L	1.31 ± 0.02	0.95 ± 0.01
Potassium, mg/L	0.75 ± 0.01	0.44 ± 0.01
Chloride, mg/L	0.43 ± 0.01	0.27 ± 0.01
Sulphate, mg/L	5.43 ± 0.04	3.16 ± 0.03
Dissolved organic carbon, µmol/L	502.3 ± 14.9	604.17 ± 9.16
Total dissolved phosphorus, µg/L	2.0 ± 0.2	3.17 ± 0.35
Total dissolved nitrogen, µg/L	224.6 ± 4.8	273.33 ± 5.93
Chlorophyll *a*, µg/L	1.10 ± 0.07	2.02 ± 0.26
Soluble reactive silicon, mg/L	2.23 ± 0.02	0.18 ± 0.02

[a]Data averaged over May–October ($N = 13$).
[b]Data averaged over May–October ($N = 12$).

Water Sampling and Processing

Lake profiles were sampled by peristaltic pump from depths of 1, 5, 10, 15, 20, 25, and 29 m in Lake 239 on 12 June 1991. Lake 382 was sampled by the same method at depths of 1, 4, 7, 10, and 12 m on 13 June 1991. Water samples were brought to an on-site laboratory in four 500-mL polyethylene bottles for subsequent processing. Within a few hours of sampling, the water was filtered in 150-mL aliquots through preashed Whatman GF/C glass fibre filters. Eight or 10 replicate filters were prepared for each depth, one half for the chemical determination of C and N and one half for the NIRS determinations (for C, N, and P) followed by chemical analysis for P. All filters were stored individually in disposable Petri dishes and frozen until analyzed.

In addition, the epilimnia of both lakes were sampled seasonally every two weeks for a variety of chemical parameters analyzed by the methods of Stainton et al. (1977). Seasonal sampling in Lake 239 was conducted by taking an integrated sample from the photic zone, which represented epilimnion and metalimnion, and from the hypolimnion.

Chemical Determination of Particulate C, N, and P

Determination of particulate C, N, and P was performed by the Freshwater Institute Analytical Laboratory using modifications of the methods of Stainton et al. (1977). The analysis of C and N involved the combustion of the filter papers in an oxygen–helium atmosphere at 950°C. The combustion products were then swept by helium carrier gas over a heated copper oxide – silver vanadate bed where they were oxidized to CO_2, H_2O, and the oxides of N. The gas stream then passed over hot copper where oxides of N were reduced to N_2 and excess O_2 was removed. CO_2 and N_2 were then measured by thermal conductance. The concentration of C or N in micrograms per litre of lake water was calculated from the mass of CO_2 or N_2 measured per GF/C filter, knowing the volume of lake water passed through that filter.

Particulate P was determined by combusting the organic matter at 500°C and then hydrolyzing the phosphorus oxides to PO_4 with HCl. Phosphate was then measured using automated acid molybdate colorimetry (Stainton et al. 1977).

The precision (coefficient of variability) for the determination of C at 1000 µg/L, N at 140 µg/L, and P at 25 µg/L is 3, 4.3, and 6%, respectively.

Determination of Particulate C, N, and P using NIRS

Fifty-three filters, subsequently used for the chemical determination of P, were dried at 60°C for 2–3 h and scanned in a NIRSystems model 6500 visible/NIR spectrophotometer over a wavelength range from 400 to 2500 nm. A filter that had been saturated with distilled water and dried under the same conditions as the 53 test filters was used as a reference for the NIRS scans. A similar technique has been reported by Meurens et al. (1987).

The spectra from two thirds (35) of the filters, representing the full range of concentrations of suspended material, composed the calibration set. Calibrations were developed over wavelengths of 400–1100, 400–1800, and 1100–2500 nm. The most successful range was 400–1800 nm. This is a region that has recently come under investigation as having the potential for making measurements in both the visible and NIR areas. Calibration equations were computed using the raw optical data smoothed over four and 10 wavelength points (log $1/R$) and using the first and second derivative of the log $1/R$ data, with several combinations of segment (smoothing) and derivative ("gap") sizes. Using the calibration equations, the concentrations of particulate C, N, and P were predicted from the validation file which contained the remaining third of the spectra ($N = 18$).

TABLE 2. Concentrations (mean ± SE) of particulate C, N, and P in lake water from several depths in two Precambrian Shield lakes determined by wet chemistry. For Lake 239, $N = 4$ per depth and for Lake 382, $N = 5$ per depth.

Date of sampling	Lake	Depth, m	Particulate C, µg/L	Particulate N, µg/L	Particulate P, µg/L
12 June 1991, day 163	239	1	422.5 ± 17.8	42.0 ± 2.7	3.50 ± 0.25
		5	517.5 ± 15.2	41.5 ± 4.0	3.75 ± 0.25
		10	552.5 ± 27.7	50.0 ± 6.3	3.50 ± 0.25
		15	327.5 ± 26.6	54.0 ± 5.7	3.75 ± 0.22
		20	302.5 ± 11.4	58.0 ± 3.1	3.50 ± 0.41
		25	332.5 ± 12.4	64.0 ± 4.0	3.75 ± 0.41
		29	565.0 ± 11.5	62.3 ± 3.5	4.00 ± 0.35
13 June 1991, day 164	382	1	538.0 ± 17.8	60.4 ± 1.6	3.80 ± 0.33
		4	594.0 ± 25.7	57.8 ± 5 .2	3.60 ± 0.22
		7	756.0 ± 24.1	79.2 ± 6.1	6.60 ± 0.61
		10	1322.0 ± 22.2	162.8 ± 3.1	9.40 ± 0.22
		12	1310.0 ± 19.8	161.0 ± 2.3	12.75 ± 0.49

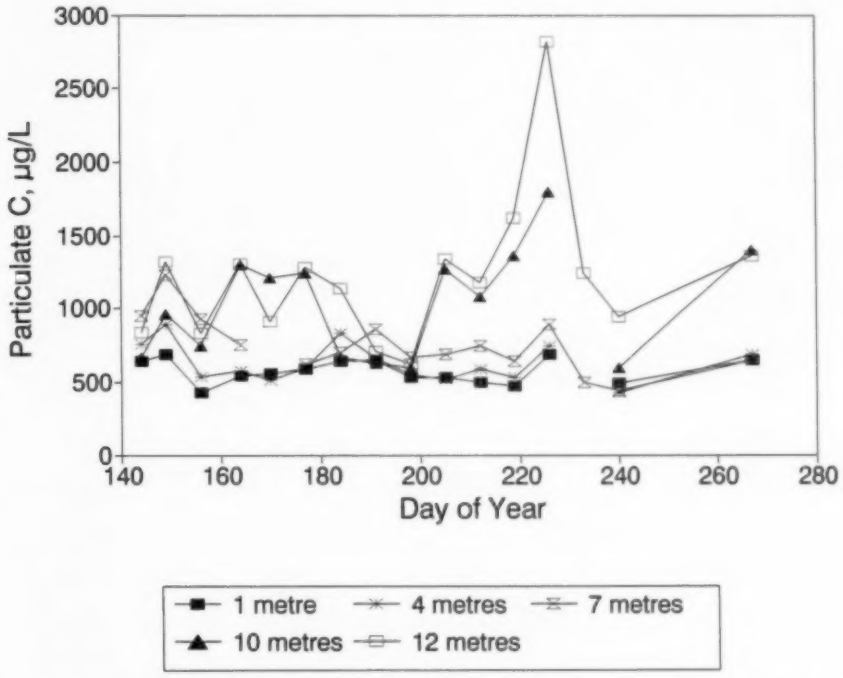


FIG. 1. Concentrations of particulate C (measured by wet chemistry) during the ice-free season of 1991 in Lake 382 at five depths. Samples measured by NIRS in this study were from day 164.

Results

Chemically Determined Particulate C, N, and P

In mid-June, particulate C was from 5 to 12-fold the concentration of particulate N which in turn was 12–17 times the particulate P (Table 2). Concentrations of all three parameters tended to increase with water depth in Lake 382, but generally not in Lake 239 (Table 2). Concentrations were higher in Lake 382 than in Lake 239.The seasonal pattern of particulate N (not shown) was very similar to that of particulate C in Lake 382 (Fig. 1). Concentrations of particulate C (Fig. 1), N, and P (Fig. 2) at 1 and 4 m depths and particulate C and N at 7 m depth were relatively constant over the ice-free season in Lake 382. The concentrations of these three parameters at 10 and 12 m depths reached maxima in mid-August. Thus, the samples of particulate C, N, and P at 1, 4, and 7 m depths from 13 June measured in Lake 382 were representative of concentrations over the entire ice-free season whereas the samples from 10 and 12 m depths were less representative.

The seasonal patterns of particulate C (Fig. 3) and N (not shown) in Lake 239 were similar to each other. For both parameters, the values for the upper, photic zone were higher than for the hypolimnion. For particulate P (not shown), values were higher in the photic zone until late June after which they were lower in the photic zone than in the hypolimnion.

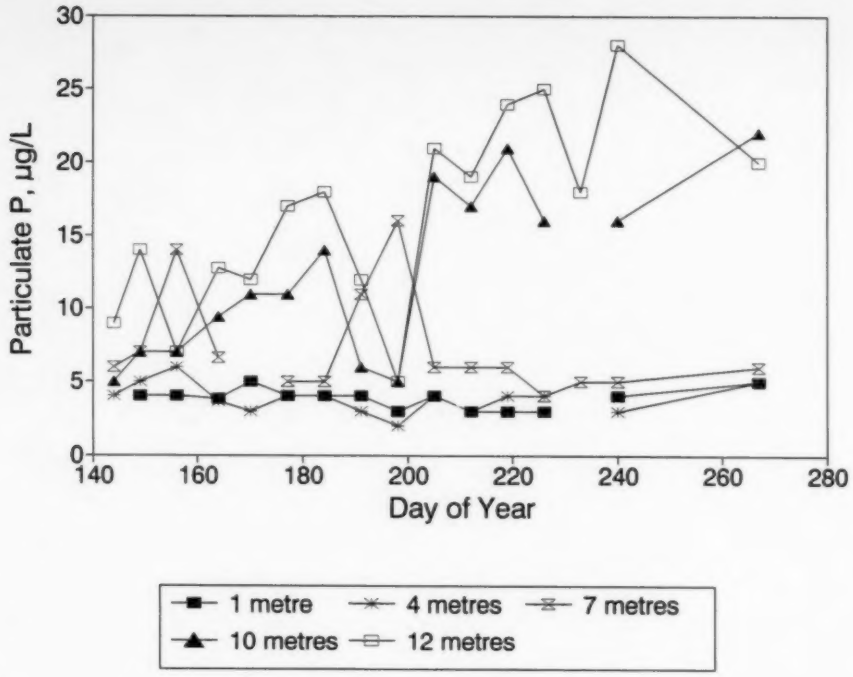


FIG. 2. Concentrations of particulate P (measured by wet chemistry) during the ice-free season of 1991 in Lake 382 at five depths. Samples measured by NIRS in this study were from day 164.

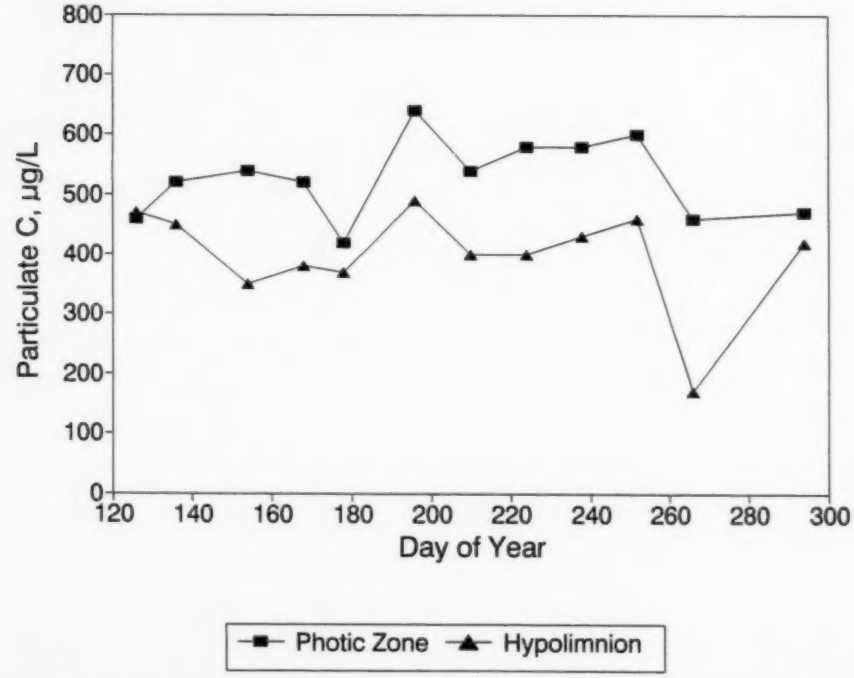


FIG. 3. Concentrations of particulate C (measured by wet chemistry) during the ice-free season of 1991 in Lake 239 in the photic zone and the hypoliminion.

TABLE 3. Accuracy of prediction of particulate C, N, and P in lake water.

Statistic	C		N		P
	Full range	Restricted range	Full range	Restricted range	Full range
r^2	0.982	0.933	0.988	0.913	0.966
SEP[a]	48.0	39.9	4.91	4.28	0.59
RDP[b]	7.52	3.57	9.51	4.11	5.37
Low	280	280	31	31	3
High	1390	840	174	105	14
Mean	668	495	78	56	5.1
SD	361.6	142.4	46.7	17.6	3.17

[a]Standard error of prediction.
[b]Ratio of SD to SEP (SD/SEP).

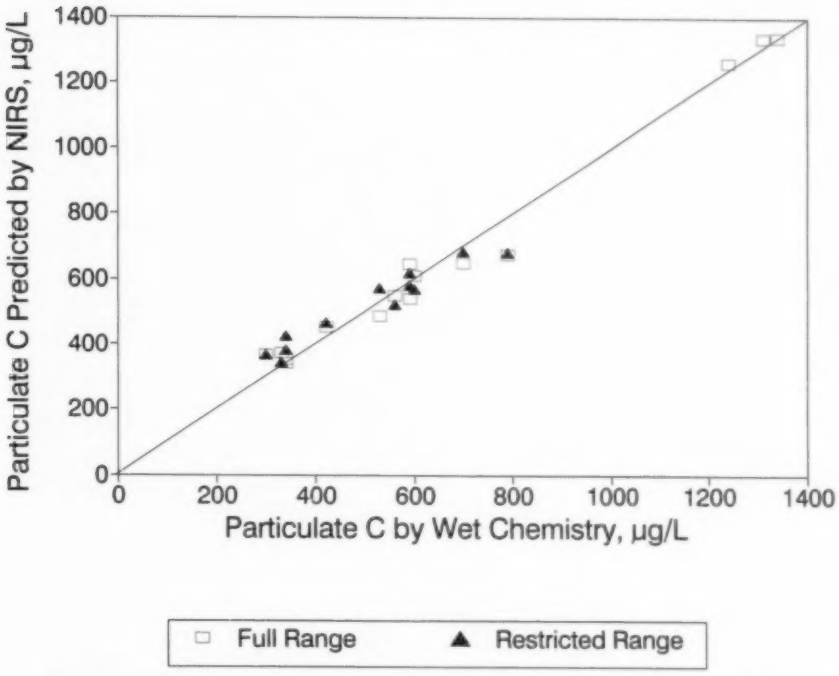


FIG. 4. Relationship between concentrations of particulate C for mid-June 1991 in Lakes 239 and 382 predicted by NIRS and measured by wet chemistry. The line is 1:1.

Prediction of Particulate C, N, and P using NIRS

Lake 382 exhibited greater ranges in and higher average concentrations of particulate C, N, and P than Lake 239 (Table 2). C and N concentrations were not continuous over their ranges. Therefore, calibrations were developed from pooled data from both lakes for both the full range (C, 280–1390 µg/L; N, 31–174 µg/L) and for the restricted range (C, 280–840 µg/L; and N, 31–105 µg/L). P was more evenly distributed, and a single calibration was developed to service the samples from both lakes. Table 3 summarizes the efficiency of all calibrations developed for particulate C, N, and P.

The RDP statistic (ratio of the standard deviation (SD) of the original data to the standard error of prediction, SEP) was introduced in 1987 to standardize evaluation of the efficiency of NIRS calibrations (Williams 1991). Standard errors of prediction can vary widely, depending upon the parameters being measured. For example, in the present study the SEP figures range from 48 to 0.6 (Table 3). The ratio of the SD of the original data to the SEP should be as high as possible. If the two statistics are equal, i.e., SD = SEP, or RDP = 1, then the calibration is not capable of differentiating clearly between any two individual samples. RDPs of 3.0 and over are suitable for screening in plant breeding programmes and for process control. For the most accurate analysis, RDPs of 5.0 and over are required.

The NIRS predictions closely matched the standard wet chemical determinations of particulate C concentrations in the total and restricted sets of data (Fig. 4). These relationships are representative of those for N and P (Table 3).

Table 4 summarizes the mathematical treatments and wave-

TABLE 4. Mathematical treatments and wavelengths (up to six) used in prediction of particulate C, N, and P in full and restricted range sample sets. Wavelength range is 400–1800 nm.

	C		N		P
	Full range	Restricted range	Full range	Restricted range	Full range
Math.[a]	D10D	D20D	D10D	D20D	log 1/*R*
Seg./Gap[b]	2/12	2/12	6/2	2/12	10
1	PLS[c]	424	688	688	472
2	—	1408	1480	712	400
3	—	712	712	1480	—
4	—	688	448	1432	—
5	—	—	880	1384	—
6	—	—	1432	448	—

[a]Mathematical treatments: D10D = first derivative and D20D = second derivative of log 1/R.
[b]Segment = degree of smoothing; gap = derivative size or distance in wavelength points between the beginning and end of the derivative.
[c]Partial least squares treatment uses all the available wavelengths.

lengths used in the development of the NIRS calibration equations. First- or second-derivative treatments of the log 1/*R* optical signals are effective in correcting for variance due to differences in mean particle size and particle size distribution among individual samples (Norris and Williams 1984). Principal component/partial least squares (PCA/PLS) regressions employ all wavelengths. A PCA/PLS equation may contain up to 15 factors (using the software provided by the instrument manufacturers).

Data are presented only for the best equations. The wavelengths selected for the equations for prediction of particulate N in the full and restricted range sample set were similar, and three of the four wavelengths selected by conventional regression for suspended C in the full range sample set (not shown because the PCA/PLS equation was superior) were the same as those used in the conventional D20D equation used for the restricted range sample set.

For the full range sample sets, the coefficients of correlation between C and N, C and P, and N and P were, respectively, 0.993, 0.914, and 0.906. For the restricted range, the corresponding figures were 0.954, 0.646, and 0.610.

Discussion

NIRS has been used widely for the quantitative determination of complex organic molecules such as proteins, amino acids, oils, and carbohydrates. We have demonstrated that it is feasible, within the ranges represented by the samples here, to measure particulate C, N, and P using NIRS in the water column of oligotrophic lakes. The samples, ranging from 420 to 2900 µg/L suspended solids, were from a variety of depths in two lakes at a mid-June date. This range reflects most of the quantitative seasonal change in suspended matter for these two lakes. Suspended matter in lakes changes not only quantitatively but also qualitatively over the seasons and may also vary from one lake type to another with pH, trophic status, humic matter content, and pollution load. The wider applicability of the NIRS technique needs to be tested by examining the relationship between NIRS-predicted and chemically measured concentrations of particulate C, N, and P over the entire year from a wider variety of lake types.

As outlined by Williams (1991), there are precautions to be observed when using the method. The analysis is sensitive to the water content of the sample. If the samples are presented on filters, as in this study, it is essential that the filters be dried to contain a consistent, low amount of moisture. The accuracy of the method is limited by that of the analytical methods used for the calibration. It is necessary in the determination of parameters by NIRS that test samples exhibiting a range of composition be analyzed periodically to verify the calibration and maintain accuracy. The calibration must be repeated when the nature of the samples being analyzed is different from those originally analyzed. For example, measurements of particulate C, N, and P in seawater would require a separate calibration.

In summary, NIRS is a rapid, low-cost, nonchemical, low environmental impact, nondestructive, efficient method for the analysis of C, N, and P in organic matter from a variety of sources, including suspended matter in oligotrophic lakes.

Acknowledgments

M. Holoka and D. Tenkula collected the water samples from the field. D. Tenkula and P.S.S. Chang assisted with laboratory processing of samples, and H. Mackling performed data analysis. The manuscript was reviewed by G. Stern and S. Lawrence.

References

ARMSTRONG, F.A.J., AND D.W. SCHINDLER. 1971. Preliminary chemical characterization of waters in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 171–187.

BIERMAN, V.J. JR., AND W.R. SWAIN. 1982. Mass balance modeling of DDT dynamics in Lakes Michigan and Superior. Environ. Sci. Technol. 16: 572–579.

BRUNSKILL, G.J., AND D.W. SCHINDLER. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 139–155.

EISENREICH, S.J., AND G.J. HOLLOD. 1979. Accumulation of polychlorinated biphenyls (PCBs)in surficial Lake Superior sediments. Atmospheric deposition. Environ. Sci. Technol. 13: 569–573.

LUM, K.R. 1987. Cadmium in fresh waters: the Great Lakes and St. Lawrence River, p. 35–50. *In* J.O. Nriagu and J.B. Sprague [ed.] Cadmium in the aquatic environment. Adv. Environ. Sci. Technol. Vol. 19. Wiley-Interscience, New York, N.Y.

MALLEY, D.F. ET AL. 1991. Biological effects of cadmium on a small Precambrian Shield Lake: Is the Canadian water quality guideline safe? p. 40–46. *In* P. Chapman, F. Bishay, E. Power, K. Hall, L. Harding, D. McLeay,

M. Nassichuk, and W. Knapp [ed.] Proceedings of the Seventeenth Annual Aquatic Toxicity Workshop, 5–7 November 1990, Vancouver, B.C. Can. Tech. Rep. Fish. Aquat. Sci. 1774. Vol. 1.

MALLEY, D.F., P.S.S. CHANG, AND R.H. HESSLEIN. 1989. Whole lake addition of cadmium-109: radiotracer accumulation in the mussel population in the first season. Sci. Total Environ. 87/88: 397–417.

MATHIAS, J.A., P.C. WILLIAMS, AND D.C. SOBERING. 1987. The determination of lipid and protein in freshwater fish using near-infrared reflectance spectroscopy. Aquaculture 61: 303–311.

MEURENS, M., P. VAN DEN EYNDE, AND M. VANBELLE. 1987. Fine analysis of liquids by NIR reflectance of dry extract on solid support (DESIR), p. 297–302. *In* J. Holló, K.J. Kaffka, and J.L. Gönczy [ed.] Near-infrared diffuse reflectance/transmittance spectroscopy. Proceedings of the International NIR/NIT Conference, 12–16 May 1986, Budapest, Hungary. Akademiai Kiado, Budapest.

NORRIS, K.H., AND P.C. WILLIAMS. 1984. Optomization of mathematical treatments of raw near-infrared signal in the measurement of protein in hard red spring wheat. I. Influence of particle size. Cereal Chem. 61: 158–165.

OSBORNE, B.G., AND T. FEARN.1986. Near-infrared spectroscopy in food analysis. Longmans, New York, N.Y.

PROKOPOWICH, J. 1979. Chemical characterization of epilimnion waters in the Experimental Lakes Area, northwestern Ontario. Can. Fish. Mar. Serv. Tech. Rep. 873: iv + 41 p.

SANDOVAL, M., F.H. VERHOFF, AND T.H. CAHILL. 1976. Mathematical modeling of nutrient cycling in rivers, p. 203–231. *In* R.P. Canale [ed.] Modeling biochemical processes in aquatic ecosystems. Ann Arbor Science Publishers Inc., Ann Arbor, Mich.

STAINTON, M.P., M.J. CAPEL, AND F.A.J. ARMSTRONG. 1977. The chemical analysis of fresh water. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25: 180 p.

WETZEL, D.L. 1983. Near-infrared reflectance analysis: sleeper in analytical techniques. Anal. Chem. 55: 1165a–1176a.

WILLIAMS, P. 1991. Chemical fundamentals of near-infrared technology, p. 159–180. *In* L.D. Hill [ed.] Uniformity by 2000: highlights of an International Workshop on Maize and Soybean Quality. University of Illinois, Urbana, Ill.

WILLIAMS, P.C., AND K.H. NORRIS. 1987. Near-infrared technology in the agricultural and food industries. American Association of Cereal Chemists Inc., St. Paul, Minn. 330 p.

Influence of the Size of Enclosures on the Swimming Characteristics of Juvenile Brook Trout (*Salvelinus fontinalis*)

Ming Tang and Daniel Boisclair

Université de Montréal, Département de sciences biologiques, C.P. 6128, Succursale « A », Montréal, QC H3C 3J7, Canada

and

Station de biologie des Laurentides de l'Université de Montréal, 440, avenue du Lac Croche, C.P. 159, St-Hippolyte, QC G0R 1P0, Canada

Tang, M., and D. Boisclair. 1993. Influence of the size of enclosures on the swimming characteristics of juvenile brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 50: 1786–1793.

We tested the hypothesis that swimming characteristics of an active planktivorous fish, brook trout (*Salvelinus fontinalis*), are significantly influenced by the size of enclosure and the time of day. Swimming characteristics of 0+ brook trout kept in 1,- 8,- and 27-m^3 enclosures were recorded with an underwater videocamera system between 06:00 and 20:00. Enclosure size had a significant influence on swimming characteristics. Median speed (range = 6.4–11.1 cm/s) and associated variance observed in the 27-m^3 enclosure were approximately twice those estimated in the 1- and 8-m^3 enclosures. Variance of acceleration rates varied three- to fourfold among enclosures. Median turning rates (range = 11.8–19.8°/s) and corresponding variances tended to decrease as enclosure size increased. Our analyses suggest that models of spontaneous swimming costs developed using respirometry experiments performed in small aquaria may not appropriately represent the complexity of swimming patterns, and consequently, the costs of spontaneous swimming in large enclosures or in the field.

Nous avons testé l'hypothèse que les caractéristiques de nage d'un poisson planctivore actif, l'omble de fontaine (*Salvelinus fontinalis*), sont significativement influencées par la taille des enclos et l'heure du jour. Les caractéristiques de nage d'ombles de fontaine 0+ gardés dans des enclos de 1, 8, et 27 m^3 ont été enregistrées avec des vidéo-caméras sous-marines entre 06:00 et 20:00. La taille des enclos a eu une influence significative sur les caractéristiques de nage. La vitesse médiane (étendue = 6,4–11,1 cm/s) et la variance observées dans l'enclos de 27 m^3 ont été près du double des valeurs estimées dans les enclos de 1 et 8 m^3. Les variances des taux d'accélération ont varié par un facteur de 3 à 4 entre les enclos. Les médianes des taux de virage (étendue = 11,8–19,8°/s) et les variances correspondantes ont diminué avec l'augmentation de la taille des enclos. Nos analyses suggèrent que les modèles des coûts de nage spontanée dérivés d'expériences respirométriques exécutées dans de petits aquaria peuvent ne pas représenter la complexité des patrons de nage, et par conséquent, les coûts de la nage spontanée dans de grands enclos ou sur le terrain.

Received August 11, 1992
Accepted March 9, 1993
(JB596)

Reçu le 11 août 1992
Accepté le 9 mars 1993

Laboratory (Koch and Wieser 1983; Hart and Connellan 1984) and field experiments (Boisclair and Leggett 1989) as well as theoretical models (Kerr 1971, 1982) suggest that the quantity of energy allocated to activity has a strong influence on the quantity of energy available for fish growth and reproduction. However, no direct test of this hypothesis has been performed, mainly because of the difficulty of estimating fish activity costs.

The most common strategy to estimate fish activity cost under field situations involves the estimation of swimming speed and its transformation into energy expenditure (Feldmeth and Jenkins 1973; Ware 1975, 1978; Puckett and Dill 1985; Boisclair 1992b). The transformation of swimming speed into energetic cost is often based on laboratory-derived relationships between oxygen consumption and speed for fish swimming against a unidirectional current of known velocity (forced swimming; Brett 1965, 1973; see Beamish 1978 for a review). Experimental and empirical studies indicate that oxygen consumption – speed relationships derived using the forced swimming approach may underestimate true costs associated with swimming at a given speed under natural conditions by a factor ranging from 3 to 20 (Beamish and Dickie 1967; Puckett and Dill 1984; Weatherley and Gill 1987; Webb 1991). This difference has been attributed to the costs of performing turns and accelerations which are not considered during forced swimming experiments (Forstner and Wieser 1990; Boisclair and Tang 1993). One alternative approach has been to use oxygen consumption – speed relationships based on free-swimming fish under laboratory conditions (spontaneous swimming: Muir et al. 1965; Smit 1965; Wohlschlag and Cameron 1967; Kutty 1969; Muir and Niimi 1972; Durbin et al. 1981; Weatherley et al. 1982) to transform field-derived speed values in energetic cost. However, most laboratory experiments on spontaneous swimming have been performed in relatively small respirometers that may not allow fish to display acceleration and turning rates adopted in large enclosures or in the field, and consequently, may not adequately represent corresponding spontaneous swimming costs. Assessment of the magnitude of the bias introduced by using laboratory-derived oxygen consumption – spontaneous swimming speed relationships to estimate activity costs in large enclosures or in the field requires (1) the evaluation of the influence of enclosure size on swimming characteristics, (2) the

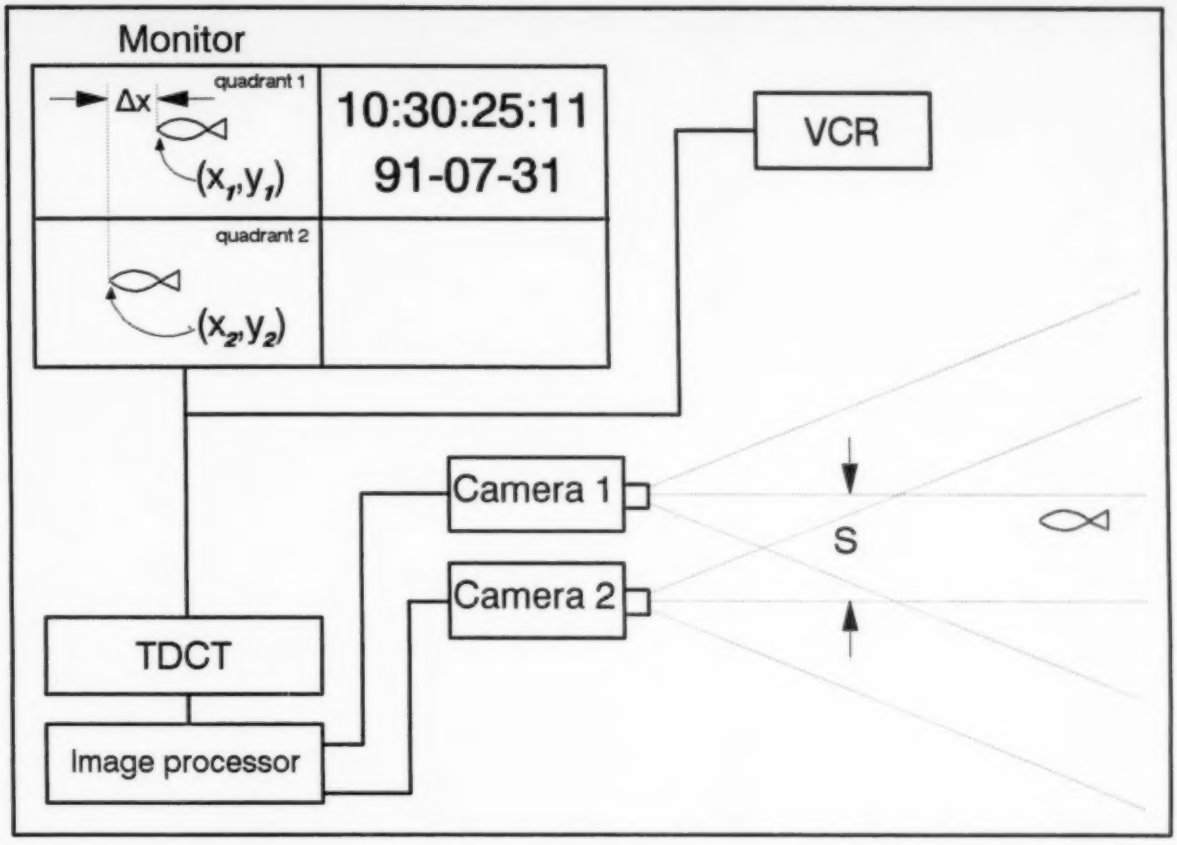


FIG. 1. Videocamera system. Images perceived by two cameras are combined and synchronized by an image processor, the camera titler (TDCT) copies the time on the images (hours-minutes-seconds), and these images are recorded using a videocamera recorder (VCR). Characteristics such as optical axes separation (S), coordinates of the targets as they appear on different quadrants of the monitor (x_1,y_1 and x_2,y_2), and the apparent target displacement (Δx) are used to estimate fish coordinates in three dimensions (x,y,z).

description of fish swimming characteristics under field situations, and (3) the estimation of the influence of variations of swimming characteristics on energy consumption. However, no quantitative analysis of the influence of enclosure size on swimming characteristics and no data on these characteristics under field situations are available. Consequently, it is presently impossible to evaluate the swimming characteristics that should be simulated under laboratory conditions to adequately represent spontaneous swimming costs in enclosures or in the field.

Our purpose is to quantify the influence of the volume of enclosures on fish swimming characteristics. We also present data that may illustrate the range of movements executed by an active planktivorous fish (relative to a filter feeder) in the field.

Methods

Field Experiments

Experiments designed to evaluate the influence of enclosure size on fish swimming characteristics were performed using 1-, 8-, and 27-m^3 cubic enclosures. Mesh size (0.06 cm^2) allowed zooplankton to enter or exit the enclosures. Enclosures were moored on the 3-m depth isobath of Lake Croche, a mesotrophic lake located at La Station de Biologie des Laurentides de l'Université de Montréal (70 km northwest of Montréal), in the Lower Laurentians region of Québec. One experiment was performed in each enclosure. These experiments are hereafter referred to as Exp 1 (1 m^3; July 31, 1991), 2 (8 m^3; August 13, 1991), and 3 (27 m^3; August 15, 1991).

Five young-of-the-year brook trout (*Salvelinus fontinalis*; mean total length = 8.08, SD = 0.46; mean net weight = 5.05 g SD = 2.01) were introduced into the enclosure 1 d before each experiment to allow the fish to recuperate from manipulation and stress associated with their transfer (Boisclair 1992b). Fish swimming characteristics were observed and recorded using an underwater videocamera system (Boisclair 1992a). A detailed account of the possibilities and of the limitations of this method has been presented by Boisclair (1992a). Two approximately parallel-oriented cameras were placed in a waterproof Plexiglas case and installed inside the enclosure immediately following the introduction of the fish. The comparison of the movements executed by fish inside our enclosures is subjected to the assumtpion that, for each experiment, the movements observed by our cameras accurately represented the full range of move-

ments executed by fish in the entire enclosure. The cameras were therefore positioned to observe a part of the enclosure where fish swimming patterns included those far from and close to the sides of the enclosures. In this sense, we believe that the volume observed by our cameras represented an unbiased sample of the movements executed by fish in these enclosures. The cameras sampled 24% of the total volume of the 1- and 8-m^3 enclosures and 11% of the total volume of the 27-m^3 enclosure. For each experiment, fish were filmed during four 1-h periods distributed at regular intervals between 06:00 and 20:00 (06:00–07:00, 10:00–11:00, 14:00–15:00, and 19:00–20:00). Light was determined only at atmospheric conditions (50% cloudiness during the three experiments; no artificial lights were used).

Videocamera System

Swimming characteristics were derived from variations of fish position in an x,y,z cartesian coordinate system over time. Time series of the x,y,z position of the fish observed were obtained using the stereocinematographic method (Boisclair 1992a). The two perspectives of a given fish required to estimate its positions in three dimensions were provided by two Panasonic WV-BL602 cameras arranged in parallel 10 cm apart (center to center). Each camera was mounted with Cosmicar automatic iris EX-2 lenses (6 mm, F1:1.2, angle of vision: 56° horizontally, 46.3° vertically). A videoprocessor (Panasonic Quad WJ-450) was used to combine images received simultaneously from the two cameras and to present these images in adjacent quadrants on a monitor (Fig. 1). Time (hours-minutes-seconds) and date were printed by a time and date camera titler (TDCT). Final images were recorded by a Sony CCD-F70 8-mm Handycam.

Calibration of the Videocamera System

The positioning of targets in three dimensions requires the estimation of the distance that separates the optical axes of the two cameras (hereafter referred to as optical axes separation, S; Fig. 1). However, because the optical axes are rarely perfectly parallel, optical axes separation generally varies with the distance between the targets and the cameras (Van Sciver 1972; Klimley and Brown 1983; Ménard 1991). This bias can be corrected by using the relationship between optical axes separation and apparent displacement of the targets (Δx; Fig. 1). We estimated the relationship between S and Δx under field conditions by filming four rulers measuring 5, 7.5, 10, and 12 cm at distances of 25, 50, 75, 100, and 150 cm from the cameras.

Image Analysis

Images recorded were analyzed using the Jandel Video Analysis Software (Java). The position of a target (ruler or fish) was quantified using two pairs of coordinates, the x- and y-coordinates of the target in each of the two quadrants (Fig. 1). The origin (0,0) of these coordinates was the center of their respective quadrants. The units of the coordinates measured directly on the monitor of the image analyzer are hereafter identified as "cm_i". Distances estimated at the scale of the enclosures are further referred to as "cm".

The position of fish observed more than 3 consecutive seconds was estimated at 1-s intervals. When more than one fish was visible during a given time period, every fish was digitized sequentially.

Computation

We described fish swimming using four characteristics: speed, acceleration rate, turning rate, and complexity index.

Fish swimming speed V_t was defined as the average speed of the head and of the tail of a fish during a given time interval (1 s). The speed of the head (VH_t, centimetres per second) or tail (VT_t, centimetres per second) of a fish was estimated as

$$(1)\quad VH_t \text{ or } VT_t = \frac{(S_t \cdot m_t)}{\Delta \bar{x}_t}$$

where $\Delta\bar{x}_t$ (cm_i) is the average apparent displacement of the target (head or tail) between time t and $t + 1$ (Fig. 1), S_t (cm) is the optical axis separation (Fig. 1), and m_t (cm_i) is the movement of the target between t and $t + 1$ (Boisclair 1992a).

$\Delta\bar{x}_t$ was estimated as

$$(2)\quad \Delta\bar{x}_t = (\Delta x_t + \Delta x_{t+1})/2$$

where Δx_t and Δx_t+1 (cm_i) are the absolute differences between the x-coordinates of the target on both quadrants ($|x_1 - x_2|$; Fig. 1) at time t and $t + 1$, respectively.

S_t was estimated using the relationship derived during the calibration procedure: $S_t = -0.11\cdot(\Delta x_t)^2 + 1.26\cdot\Delta x_t + 5.00$ ($F_{1,22} = 104.91$; $p < 0.0001$; $R^2 = 0.91$).

m_t was estimated as

$$(3)\quad m_t = \sqrt{[(\bar{x}_{t+1} - \bar{x}_t)^2 + (\bar{y}_{t+1} - \bar{y}_t)^2 + (\bar{z}_{t+1} - \bar{z}_t)^2]}$$

where $\bar{x}$, $\bar{y}$, and $\bar{z}$ (cm_i) are the average values of the target coordinates relative to the center of the two quadrants displayed on the monitor of the image analyzer at time t and $t + 1$ (Fig. 1). The $\bar{x}$-, $\bar{y}$-, and $\bar{z}$-coordinates were determined at 1-s intervals using a modified version of the algorithm developed by Boyce (1964), Van Sciver (1972), and Long and Aoyama (1985) to estimate fish length and three-dimensional structure of fish schools.

Acceleration rates (A_t, centimetres per square second) were estimated as the difference in swimming speed of a fish between 2 consecutive seconds:

$$(4)\quad A_t = \frac{(V_{t+1} - V_t)}{\Delta t}.$$

Turning rates (D_t, degrees per second) were defined as the angle between the projection of the two lines joining the head and the tail of a fish at 2 consecutive seconds t and $t + 1$. Turning rates were estimated as

$$(5)\quad D_t = \text{Arccos}\frac{[(\bar{x}H_t - \bar{x}T_t)\cdot(\bar{x}H_{t+1} - \bar{x}T_{t+1})] + [(\bar{y}H_t - \bar{y}T_t)\cdot(\bar{y}H_{t+1} - \bar{y}T_{t+1})] + [(\bar{z}H_t - \bar{z}T_t)\cdot(\bar{z}H_{t+1} - \bar{z}T_{t+1})]}{[\sqrt{(\bar{x}H_t - \bar{x}T_t)^2 + (\bar{y}H_t - \bar{y}T_t)^2 + (\bar{z}H_t - \bar{z}T_t)^2}]\cdot[\sqrt{(\bar{x}H_{t+1} - \bar{x}T_{t+1})^2 + (\bar{y}H_{t+1} - \bar{y}T_{t+1})^2 + (\bar{z}H_{t+1} - \bar{z}T_{t+1})^2}]}$$

TABLE 1. Regression statistics of the relationship between complexity index C = untransformed data; LC = natural logarithmic transformation) and the length of observation (Δ) for the three experiments.

Exp.	Enclosure size (m^3)	df	r^2	$p > F$	Model
1	1	311	0.3322	0.0001	$LC = 0.089 \cdot \Delta - 0.078$
2	8	71	0.2898	0.0001	$LC = 0.072 \cdot \Delta - 0.072$
3	27	29	0.0738	0.1393	$C = 0.023 \cdot \Delta + 0.973$

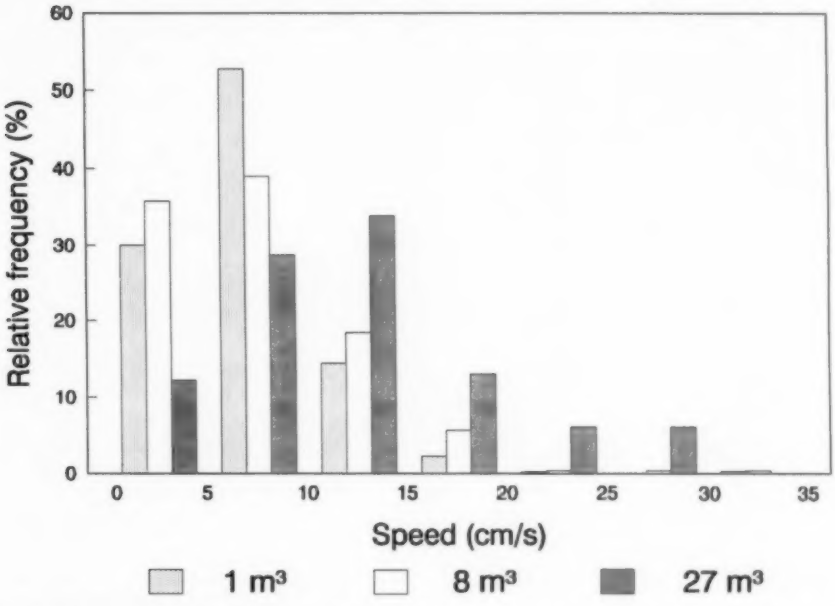


FIG. 2. Frequency distribution of fish swimming speeds in 1-m^3 (Exp. 1, $n = 1844$), 8-m^3 (Exp. 2, $n = 552$), and 27-m^3 (Exp. 3, $n = 115$) enclosures.

where $\bar{x}T$, $\bar{y}T$, and $\bar{z}T$ are, respectively, the x-, y-, and z-coordinates of the head of the fish at times t or $t + 1$ and $\bar{x}Q$, $\bar{y}Q$, and $\bar{z}Q$ are corresponding coordinates for the tail of that fish.

Complexity index was estimated as

$$(6) \quad C = M_\Delta / D_\Delta$$

where M_Δ (cm$_t$) is the sum of the movements executed by a fish during a given time interval t to $t + \Delta$ and D_Δ (cm$_t$) is the net distance covered by the fish during that interval. For any observation sequence, we only estimated the complexity index associated with the longest possible Δ.

M_Δ was estimated as

$$(7) \quad M_\Delta = \sum_{t=1}^{\Delta} m_t .$$

D_Δ was estimated as

$$(8) \quad D_\Delta = \sqrt{[(\bar{x}_{t+\Delta} - \bar{x}_t)^2 + (\bar{y}_{t+\Delta} - \bar{y}_t)^2 + (\bar{z}_{t+\Delta} - \bar{z}_t)^2]} .$$

Differences ($R\%$) between median speed, acceleration, and turning rates at different times of day within experiments were quantified using relative percentage

$$(9) \quad R\% = \frac{(\text{maximum median} - \text{minimum median}) \cdot 200}{\text{maximum median} + \text{minimum median}} .$$

Environmental Conditions Inside the Enclosures

Environmental conditions inside the enclosures were described using water temperature, oxygen concentration, and zooplankton biomass. Water temperature and oxygen concentration were measured within 5 m of the enclosures using a YSI oxygen meter (model 57). Measurements were taken at 1-m intervals from the surface to a depth of 3 m. Zooplankton was sampled using a battery-powered bilge pump (2800 L/h). Five 10-L samples were collected from the enclosures within 30 min before the beginning and 30 min after the end of each experiment. Zooplankton samples were individually filtered on 125-µm filters, dried for 2 h at 60°C, and weighed to the nearest 0.0001 g dry weight.

TABLE 2. Fish swimming speed (V_t, cm/s), acceleration rates (A_t, cm/s^2), and turning rates (D_t, degree/s) for each experiment and time of day ($\tilde{x}$ = median; s^2 = variance; n = sample size).

Exp. (enclosure size)			Time of day 06:00–07:00	10:00–11:00	14:00–15:00	19:00–20:00	Daily value
1 (1 m^3)	V_t	$\tilde{x}$	6.9	7.0	6.5	5.6	6.5
		s^2	8.4	11.4	23.7	8.1	15.7
		n	353	376	797	318	1844
	A_t	$\tilde{x}$	−0.3	−0.15	−0.26	−0.34	−0.22
		s^2	13.4	17.5	22.3	9.1	17.1
		n	266	306	689	271	1532
	D_t	$\tilde{x}$	20.7	18.5	21.3	17.4	19.8
		s^2	1041.2	1006.2	1467.3	1054.5	1203.9
		n	353	376	797	318	1844
2 (8 m^3)	V_t	$\tilde{x}$	7.6	6.8	7.5	4.6	6.4
		s^2	20.4	23.1	25.8	20.4	22.9
		n	132	136	139	145	552
	A_t	$\tilde{x}$	0.02	−0.32	−0.26	0.27	0.25
		s^2	16.8	19.8	14.2	13.5	15.2
		n	114	119	118	128	479
	D_t	$\tilde{x}$	17.2	16.2	13.8	11.6	14.1
		s^2	1613.9	1708.1	1339.9	1011.3	1387.4
		n	132	136	139	145	552
3 (27 m^3)	V_t	$\tilde{x}$	13.5	9.0	12.4	9.9	11.1
		s^2	32.8	21.7	38.5	94.4	40.5
		n	7	55	46	7	115
	A_t	$\tilde{x}$	−5.76	−0.99	−1.11	−1.89	1.11
		s^2	86.2	21.3	76.7	166.1	50.7
		n	5	43	31	5	84
	D_t	$\tilde{x}$	16.0	10.5	11.9	9.7	11.8
		s^2	19.3	1242.9	179.6	145.5	681
		n	7	55	46	7	115

Data Analysis

The significance of the difference of swimming speed, acceleration rates, and turning rates among experiments and time of day was assessed using two-way analysis of variance. This approach included three steps. First, each value of a given swimming characteristic was ranked using the Blom procedure (Blom 1958). Second, the ranks were normalized using the Ψ transformation (Conover and Iman 1981). Third, the swimming characteristics were compared using parametric one-way analysis of variance on the ranked normalized values. Regression analysis indicated that complexity index (Eq. 6) increased with the length of the time interval Δ (Table 1). Consequently, complexity data were classified into two groups: complexity indices calculated for fish observed between 3 and 5 s and between 6 and 10 s (further referred to as Groups 1 and 2, respectively). The number of observations that lasted more than 10 s was not sufficient to perform any detailed analysis. The influence of enclosure size and duration of observation on swimming complexity was assessed using two-way analysis of variance. Complexity data were normalized using the Box–Cox transformation. The significance of the difference of the zooplankton biomass among experiments and between time (beginning and end of an experiment) was tested using two-way analysis of variance. Zooplankton biomass was normalized using natural logarithmic transformations.

Results

Swimming Speed

Fish swimming speed ranged from 0.3 to 32.6 cm/s (Fig. 2) and varied significantly with enclosure size ($F_{2,2515} = 51.27$; $p < 0.0001$) and time of day ($F_{3,2515} = 32.69$; $p < 0.0001$). The interaction term was also significant ($F_{6,2515} = 7.93$; $p < 0.0001$).

Median speeds observed in the smaller enclosures (Experiments 1 (6.5 cm/s) and 2 (6.4 cm/s)) were, on average, 1.7 times smaller than that found in the largest enclosure (Experiment 3 (11.1 cm/s)) (Table 2). Furthermore, the variances associated with the speed frequency distribution were also significantly different ($p < 0.001$) among enclosures. The variance of Experiment 3 ($s^2 = 40.5$) was 1.8–2.6 times larger than those of Experiments 2 ($s^2 = 22.9$) and 1 ($s^2 = 15.7$), respectively.

Within-day variations of median speed did not follow any well-defined pattern (Table 2). Differences among median speeds observed during different periods of the day increased from 22.3% for Experiment 1 to 48.9 and 39.9% for Experiments 2 and 3, respectively. We found a 1.3-fold (Experiment 2) to 2.9-fold (Experiment 1) difference among variances of swimming speed estimated during different periods of the day (Table 2).

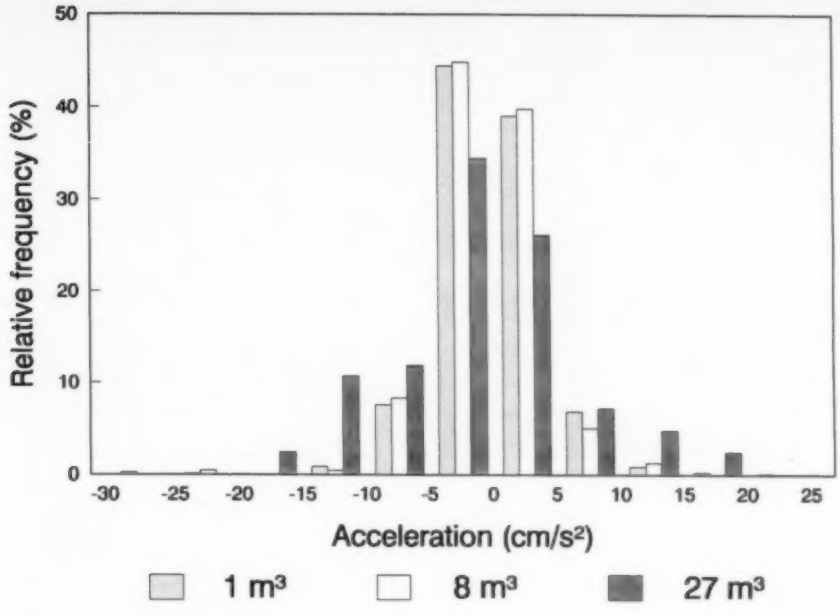


FIG. 3. Frequency distribution of fish acceleration rates in 1-m^3 (Exp. 1, $n = 1532$), 8-m^3 (Exp. 2, $n = 479$), and 27-m^3 (Exp. 3, $n = 84$) enclosures.

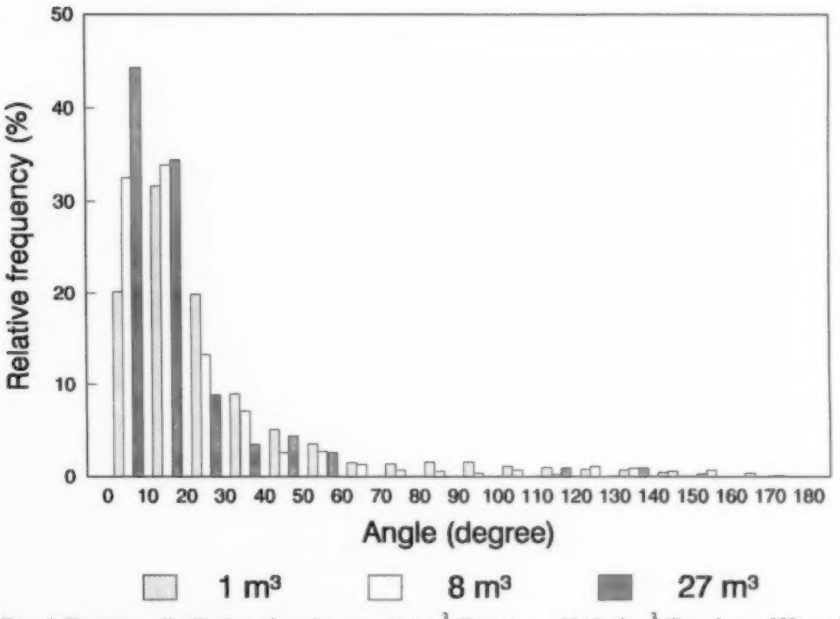


FIG. 4. Frequency distribution of turning rates in 1-m^3 (Exp. 1, $n = 1844$), 8-m^3 (Exp. 2, $n = 552$), and 27-m^3 (Exp. 3, $n = 115$) enclosures.

Acceleration Rate

Acceleration rates ranged from −26.76 (deceleration) to 23.78 cm/s^2 (Fig. 3). Enclosure size had a statistically significant influence on acceleration rates ($F_{2,2096} = 3.44$; $p = 0.04$) and associated variances ($p < 0.001$). No such influence was detected with time of a day ($F_{3,2096} = 0.52$; $p > 0.67$). The interaction term was not significant ($F_{6,2096} = 0.07$; $p > 0.99$).

Median acceleration rates were always close to 0 cm/s^2

(Table 2). Variances of acceleration rates observed during Experiments 1 ($s^2 = 17.1$) and 2 ($s^2 = 15.2$) were, on average, 3.1 times smaller than that of Experiment 3 ($s^2 = 50.7$). Furthermore, variance of acceleration rates varied 1.5-fold (Experiment 1) to 3.6-fold (Experiment 3) among different periods of the day (Table 2). Two time periods of Experiment 3 (06:00–07:00 and 19:00–20:00) were excluded from these comparisons because of low sample sizes caused by poor visibility ($n < 10$; Table 2).

Turning Rate

Turning rates ranged from 1.29 to 178.73°/s (Fig. 4). Enclosure size ($F_{2,2515} = 33.86$; $p < 0.0001$) and time of day ($F_{3,2515} = 6.15$; $p < 0.001$) had a significant influence on turning rates. The interaction term was also significant ($F_{6,2515} = 2.23$; $p < 0.05$).

Median turning rates decreased from 19.8°/s (Experiment 1) to 11.8°/s (Experiment 3) as enclosure size increased (Fig. 4; Table 2). Variances of turning rates estimated for Experiments 1 ($s^2 = 1204$) and 2 ($s^2 = 1387$) were, on average, 1.9 times larger than the corresponding value for Experiment 3 ($s^2 = 681$; Table 2), but these differences were not statistically significant ($p = 0.06$).

Within-day variations in turning rates did not follow any defined pattern (Table 2). Differences among median turning rates observed during different periods of the day increased with enclosure size (20.3% for Experiment 1, 38.9% for Experiment 2, and 48.6% for Experiment 3). Variance in turning rates varied 1.5-fold (Experiment 1) to 6.9-fold (Experiment 3) among different periods of the day. Time periods 06:00–07:00 and 19:00–20:00 of Experiment 3 were excluded from these comparisons because of low sample sizes ($n < 10$; Table 2).

Complexity

Complexity index ranged from 1 to 24.5 and varied significantly among enclosure sizes ($F_{2,350} = 10.39$; $p < 0.0001$) and between short (3–5 s) and long (6–10 s) observation groups ($F_{1,350} = 38.52$; $p < 0.0001$). The interaction term was not significant ($F_{2,350} = 1.19$; $p > 0.3$). Sample size was not sufficient to assess the influence of time of day. Average complexity decreased as enclosure size increased (Table 3). This effect was most evident for complexity indices of Group 2. Complexity indices also increased with the length of the time interval (Table 3). The variance of complexity indices estimated for Experiment 3 was significantly smaller ($1.07 < F_{max} < 59.5$; $p < 0.01$ for Group 1 and $16.4 < F_{max} < 185.7$; $p < 0.01$ for Group 2) than that for Experiments 1 and 2 (Table 3).

Environmental Conditions Inside the Enclosures

Water temperature adjacent to our enclosures averaged 24.4°C at the surface and 17.7°C at a depth of 3 m. Maximum water temperature difference among experiments was 2.5°C. Oxygen concentration was 9.1 mg/L at the surface and 11.9 mg/L at a depth of 3 m. Oxygen concentration did not differ by more than 2.0 mg/L among experiments. Mean zooplankton biomass inside the enclosures ranged from 0.98 mg/10 L (27-m^3 enclosure) to 1.18 mg/10 L (8-m^3 enclosure) at the beginning and from 0.28 mg/10 L (1-m^3 enclosure) to 0.65 mg/10L (8-m^3 enclosure) at the end of the experiments. Zooplankton biomass did not vary significantly among enclosures ($F_{2,24} = 1.55$; $p > 0.2$) but decreased from the beginning to the end of the experiments ($F_{1,24} = 20.71$; $p < 0.0001$). The interaction term was not significant ($F_{2,24} = 1.82$; $p > 0.18$).

TABLE 3. Comparison of complexity indices calculated for fish observed between 3 and 5 s (Group 1) and between 6 and 10 s (Group 2) during the three experiments ($\bar{x}$ = average; s^2 = variance).

Exp.	Enclosure size (m^3)	Group 1		Group 2	
		$\bar{x}$	s^2	$\bar{x}$	s^2
1	1	1.50	1.19	2.14	5.57
2	8	1.53	1.11	1.84	0.34
3	27	1.07	0.02	1.11	0.03

Our analyses of water temperature, oxygen concentration, and zooplankton biomass suggest that the three experiments were performed under similar environmental conditions.

Discussion

Our analyses indicate that enclosure size and time of day have a statistically significant influence on fish swimming characteristics. Movements tended towards faster and more linear patterns as enclosure size increased. The small enclosure (1 m^3) apparently restricted fish movements and forced them to execute sharper and more frequent turns. In contrast, the space provided by the 27-m^3 enclosure allowed fish to attain faster speeds and acceleration rates. The influence of the size of an aquarium (or enclosure) on fish movements can be described using the swimming characteristics and, as noted by Webb (1991), the frequency of movements performed near the sides of the aquarium. In our study, the volume between the sides of the enclosure and an arbitrarily set limit of 10 cm from the sides of the 1-m^3 enclosure represented 29% of the total volume under observation. However, 40% of the movements observed in the 1-m^3 enclosure were executed within this zone. Fish therefore spent proportionally more time close to the edge of the 1-m^3 enclosure than expected following a random distribution (29%). In contrast, fish in the 8- and 27-m^3 enclosures executed fewer movements near the sides of the enclosure than expected. The volume of the layer 10 cm from the sides of the 8- and 27-m^3 enclosures corresponded, respectively, to 16 and 11% of the volume under observation. Proportion of the movements executed within that zone was 8% for the 8-m^3 and 3% for the 27-m^3 enclosures. Whether these percentages indicate that the 27-m^3 enclosure did not impose any biologically significant limit to the movement of the fish is not known and may be difficult to assess for juvenile brook trout, since their density in Laurentian lakes (maximum density ≈1 fish/1500 m^3; P. Magnan, Université du Québec à Trois-Rivières, personal communication) makes their filming practically impossible.

Most laboratory experiments performed to estimate the costs associated with spontaneous activity have been executed in relatively small aquaria. For instance, Kutty (1969) observed the spontaneous activity of 8.2-cm (fork length) mullet (*Liza macrolepis*) kept in 1-L containers. While Kutty did not present the exact dimensions of this container, we estimated that fish could not swim continuously for more than 2 body lengths (BL) from the center of the container before having to turn. Similar calculations performed using fish characteristics and experimental conditions from other spontaneous swimming experiments (Durbin et al. 1981; Forstner and Wieser 1990; Webb 1991) indicate that the size of the aquaria or enclosures generally

allowed fish to swim continuously for 1–4 BL from the center of the aquaria. Our analyses indicate that many characteristics of spontaneous swimming (median and variance of speed, variance of acceleration rates, variance of turning rates) observed in a volume allowing them to swim continuously for 12.4 BL (relative to the center of the 8-m^3 enclosure) may differ two- to threefold from characteristics observed when fish are allowed to swim for 18.6 BL (relative to the center of the 27-m^3 enclosure). Similar variations were also observed among periods of the day for given enclosure sizes. Our results suggest that if the costs associated with swimming at a given speed increase with swimming complexity (Forstner and Wieser 1990; Webb 1991; Boisclair and Tang 1993), extrapolation of laboratory-derived models of spontaneous swimming costs developed using small containers may not appropriately represent costs of spontaneous swimming in larger enclosures or in the field. The net influence of this bias on field-derived values can be expected to depend on the relative influence of the costs of executing sharper and more frequent turns (overestimation), fewer intense accelerations (underestimation), and being subjected to higher stress levels related to confinement (overestimation) in small rather than large containers.

Our study does not allow us to estimate the magnitude of the difference between the costs associated with swimming patterns observed in small and large enclosures. However, it is important to emphasize that our work does not indicate that respirometry experiments performed in small aquaria are useless and unproductive. In contrast, we believe that our study indicates that the extrapolation of respirometry experiments performed in small aquaria to estimate swimming costs under field situations may be valid under the provision that state variables (fish size, species, and water temperature) *and* fish swimming characteristics under laboratory conditions are similar to those attributes in the field. Our study further suggests that laboratory experiments designed to estimate spontaneous swimming cost of an active planktivorous fish like juvenile brook trout should simulate a range of swimming patterns similar to that presented in our Table 2. Considering the tendency of speed, variance of speed, and variance of acceleration rates to increase with enclosure size, values presented in Table 2 for the 27-m^3 enclosure should be interpreted as minimum values for field situations.

Acknowledgments

We thank Martha Krohn, Marc Trudel, François Marchand, and Jean-François Ménard for their valuable assistance in the field and laboratory. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Quebec Ministry of Education (FCAR) through grants to the Freshwater Ecology Group of the University of Montreal, and the University of Montreal through CAFIR, Research Development Funds, and NSERC (internal funds).

References

BEAMISH, F.W.H. 1978. Swimming capacity, p. 101–187. *In* W.S. Hoar and D.J. Randall [ed.] Fish physiology. Vol. VII. Academic Press, New York, N.Y.

BEAMISH, F.W.H., AND L.M. DICKIE. 1967. Metabolism and biological production in fish, p. 215–242. *In* S.D. Gerking [ed.] The biological basis of fresh water fish production. Blackwell Scientific Publications, Oxford.

BLOM, G. 1958. Statistical estimates and transformed beta variables. John Wiley & Sons, Inc., New York, N.Y. p. 1–156.

BOISCLAIR, D. 1992a. An evaluation of the stereocinematographic method to estimate fish swimming speed. Can. J. Fish. Aquat. Sci. 49: 523–531.

BOISCLAIR, D. 1992b. Relationship between feeding and activity rates for actively foraging juvenile brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 49: 2566–2573.

BOISCLAIR, D., AND W.C. LEGGETT. 1989. The importance of activity in bioenergetics models applied to actively foraging fishes. Can. J. Fish. Aquat. Sci. 46: 1859–1867.

BOISCLAIR, D., AND M. TANG. 1993. Empirical analysis of the influence of swimming pattern on the net cost of swimming in fishes. J. Fish Biol. 42: 169–183.

BOYCE, R.E. 1964. Simple scale determination on underwater stereo pairs. Deep-Sea Res. 11: 89–91.

BRETT, J.R. 1965. The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 22: 1491–1501.

BRETT, J.R. 1973. Energy expenditure of sockeye salmon, *Oncorhynchus nerka*, during sustained performance. J. Fish. Res. Board Can. 30: 1799–1809.

CONOVER, W.J., AND R.L. IMAN. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. Am. Stat. 35: 124–129.

DURBIN, A.G., E.G. DURBIN, P.G. VERITY, AND T.J. SMAYDA. 1981. Voluntary swimming speeds and respiration rates of a filter-feeding planktivore, the Atlantic menhaden, *Brevoortia tyrannus* (Pisces: Clupeidae). Fish. Bull. 78(4): 877–885.

FELDMETH, C.R., AND T.M. JENKINS. 1973. An estimate of energy expenditure by rainbow trout (*Salmo gairdneri*) in a small mountain stream. J. Fish. Res. Board Can. 30: 1755–1759.

FORSTNER, H., AND W. WIESER. 1990. Patterns of routine swimming and metabolic rate in juvenile cyprinids at three temperatures: analysis with a respirometer-activity-monitoring system. J. Comp. Physiol. B 160: 71–76.

HART, P.J.B., AND B. CONNELLAN. 1984. Cost of prey capture, growth rate and ration size in pike (*Esox lucius* L.) as functions of prey weight. J. Fish Biol. 25: 279–292.

KERR, S.R. 1971. A simulation model of lake trout growth. J. Fish. Res. Board Can. 28: 815–819.

KERR, S.R. 1982. Estimating the energy budget of actively predatory fishes. Can. J. Fish. Aquat. Sci. 39: 371–379.

KLIMLEY, A.P., AND S.T. BROWN. 1983. Stereophotography for the field biologist: measurement of lengths and three-dimensional positions of free-swimming shark. Mar. Biol. 74: 175–185.

KOCH, F., AND W. WIESER. 1983. Partitioning of energy in fish: can reduction of swimming activity compensate for the cost of production? J. Exp. Biol. 107: 141–146.

KUTTY, M.N. 1969. Oxygen consumption in the mullet (*Liza macrolepis*) with special reference to swimming velocity. Mar. Biol. 4: 239–242.

LONG, L.V., AND T. AOYAMA. 1985. Photographic measurement for obtaining the length aspect and bearing of free-swimming fish from their spatial position. Bull. Jpn. Soc. Sci. Fish. 51: 191–195.

MÉNARD, C. 1991. Utilisation de la vidéo mesurer les poissons *in situ*. M.Sc. thesis, Université de Montréal, Montréal, QC. 88 p.

MUIR, B.S., G.J. NELSON, AND K.W. BRIDGES. 1965. A method for measuring swimming speed in oxygen consumption studies on the aholehole (*Kuhlia sandvicensis*). Trans. Am. Fish. Soc. 94: 378–382.

MUIR, B.S., AND A.J. NIIMI. 1972. Oxygen consumption of the euryhaline fish aholehole (*Kuhlia sandvicensis*) with reference to salinity, swimming, and food consumption. J. Fish. Res. Board Can. 29: 67–77.

PUCKETT, K.J., AND L.M. DILL. 1984. Cost of sustained and burst swimming to juvenile coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 41: 1546–1551.

PUCKETT, K.J., AND L.M. DILL. 1985. The energetics of feeding territoriality in juvenile coho salmon (*Oncorhynchus kisutch*). Behavior 92: 97–111.

SMIT, H. 1965. Some experiments on the oxygen consumption of goldfish (*Carassius auratus* L.) in relation to swimming speed. Can. J. Zool. 43: 623–633.

VAN SCIVER, W.J. 1972. Scale determination of unrecognized undersea objects by stereographic photography. Mar. Technol. Soc. J. 6: 14–16.

WARE, D.M. 1975. Growth, metabolism, and optimal swimming speed of a pelagic fish. J. Fish. Res. Board Can. 32: 33–41.

WARE, D.M. 1978. Bioenergetics of pelagic fish: theoretical change in swimming speed and ration with body size. J. Fish. Res. Board Can. 35: 220–228.

WEATHERLEY, A.H., AND H.S. GILL. 1987. The biology of fish growth. Academic Press, New York, N.Y. 443 p.

WEATHERLEY, A.H., S.C. ROGERS, D.G. PINOCK, AND J.R. PATCH. 1982. Oxygen consumption of active rainbow trout (*Salmo gairdneri* Richardson) derived from electromyograms obtained by radiotelemetry. J. Fish Biol. 20: 479–489.

WEBB, P.W. 1991. Composition and mechanics of routine swimming of rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 583–590.

WOHLSCHLAG, D.E., AND J.N. CAMERON. 1967. Assessment of a low level stress on the respiratory metabolism of the pinfish (*Lagodon rhomboides*). Contrib. Mar. Sci. 12: 160–171.

Mercury in Sediments and Sediment Pore Water in the Laurentian Trough

Charles Gobeil

Ministère des Pêches et des Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli, QC G5H 3Z4, Canada

and Daniel Cossa

Institut Français de Recherches pour l'Exploitation de la Mer, C.P. 1049, F. 44037 Nantes, Cédex, France

Gobeil, C., and D. Cossa. 1993. Mercury in sediments and sediment pore water in the Laurentian Trough. Can. J. Fish. Aquat. Sci. 50: 1794–1800.

The concentration of dissolved mercury in the pore water of Laurentian Trough sediments varies between the detection limit (2.5 pM) and 64 pM, up to an order of magnitude enriched relative to the overlying water. Dissolved mercury is low near the sediment surface, increases with depth to 5 cm, and then decreases with further depth. Redistribution of remobilized mercury within the sediment column is, however, insufficient to explain the observed depth variations in mercury concentration. In the Lower St. Lawrence Estuary, the vertical profiles of mercury in trough sediments tend to be consistent with the chronology of mercury discharges from chlor-alkali plants located in the St. Lawrence drainage basin. The total amount of anthropogenic mercury in sediments deposited below the 200-m isobath in the entire lower St. Lawrence Estuary since the beginning of industrialization is estimated as $170 \pm 85 \times 10^3$ kg, about six times the amount of natural mercury that has accumulated during the same period. Near the seaward end of the Trough in the Gulf of St. Lawrence, sedimentary mercury concentrations are much lower than in the Estuary, with a significant portion of the anthropogenic mercury deposited in the sediments probably coming from the atmosphere.

La concentration en mercure dissous dans les eaux interstitielles des sédiments du chenal Laurentien varie entre la limite de détection (2,5 pM) et 64 pM, ce qui atteint un ordre de grandeur de plus que la teneur en mercure dans les eaux sus-jacentes. Le niveau en mercure dissous est faible près de la surface du sédiment, augmente jusqu'à la profondeur de 5 cm, puis diminue. La redistribution dans la colonne de sédiment du mercure libéré après déposition est, cependant, insuffisante pour expliquer les variations des concentrations en mercure observées selon la profondeur. Dans la partie supérieure du chenal Laurentian, dans l'estuaire maritime du Saint-Laurent, la distribution verticale des teneurs en mercure dans les sédiments tend à être cohérente avec la chronologie des déversements en mercure attribuables aux usines de chlore-alcali qui ont été situées sur le bassin versant du Saint-Laurent. Nous évaluons la quantité de mercure anthropique déposée dans les sédiments de l'estuaire maritime sous l'isobathe de 200 m depuis le début de l'industrialisation à $170 \pm 85 \times 10^3$ kg, soit environ six fois plus que la charge en mercure naturel accumulée au cours de la même période. Dans la partie inférieure du chenal, dans le golfe du Saint-Laurent, la concentration en mercure des sédiments est beaucoup plus faible que celle des sédiments de l'estuaire maritime, et une portion significative du mercure anthropique déposé dans les sédiments provient de source atmosphérique.

Received July 31, 1992
Accepted March 5, 1993
(JB588)

Reçu le 31 juillet 1992
Accepté le 5 mars 1993

Mercury profiles in sediments have often been used to document the historical evolution of mercury contamination in the aquatic environment (Klein and Goldberg 1970; Thomas 1970; Aston et al. 1973; Young et al. 1973; Smith and Loring 1981; Breteler et al. 1984). Such an interpretation of the sedimentary record assumes that the rate at which mercury is remobilized and redistributed within the sediment column is low compared with the rate at which it is deposited at the sediment–water interface. Due to a paucity of data on mercury in interstitial water, this assumption has never been adequately verified. Lindberg and Harriss (1974) found high mercury concentrations in pore water of estuarine sediments and suggested that mercury may exist as organic and polysulfide complexes in pore water. To our knowledge, the only other field study focusing on the remobilization of mercury in interstitial water was conducted by Bothner et al. (1980). These authors showed that, in a highly contaminated estuarine environment, reducing sediments may release measurable amounts of mercury to pore water and subsequently to overlying water.

Prior to government regulations implemented in the early 1970's to reduce industrial mercury contamination, chlor-alkali factories in Canada, as in many other developed countries, were a major source of mercury in fresh and marine waters. The Great Lakes and the St. Lawrence hydrological system have been particularly affected by the discharges of liquid effluents enriched in mercury from several of these plants (Cossa 1990). One of the most important sources of mercury was located on the Saguenay River, a tributary of the St. Lawrence Estuary. This plant, in operation from 1948 to 1976, was responsible for a severe case of mercury pollution that led to the closure of commercial fisheries in the Saguenay Fjord. According to Loring and Bewers (1978), some of the industrial mercury discharged into the

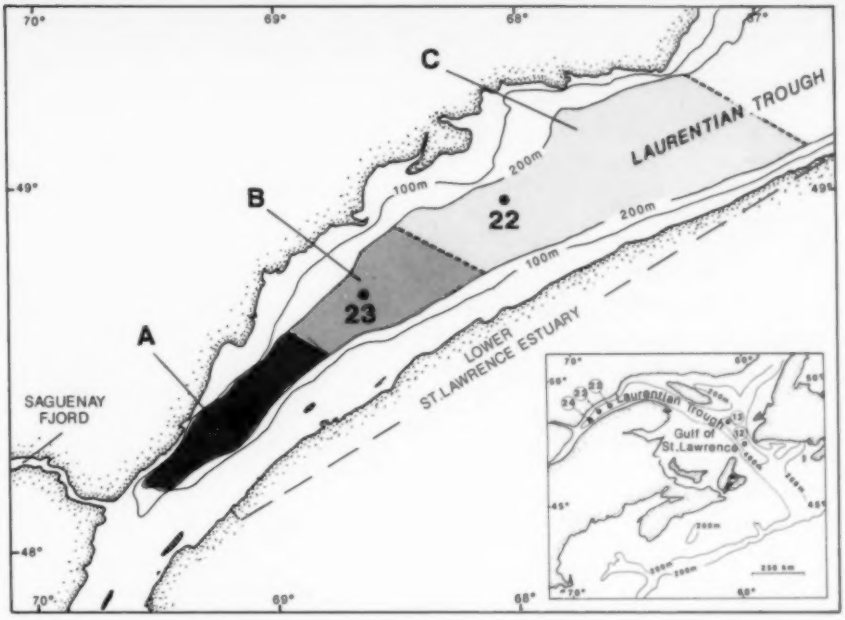


FIG. 1. Map of the Laurentian Trough showing the locations of our sampling stations in the Lower Estuary (Stations 22, 23, and 24) and Gulf of St. Lawrence (Stations 12 and 13). Zones A, B, and C are defined in the text.

Saguenay has been transported to the adjacent Lower St. Lawrence Estuary, where mercury in excess of the natural background levels has been found in the surficial sediments of the Laurentian Trough (Loring 1975).

Because it is located downstream from the industrialized and urban areas of the Great Lakes and is the principal site of sedimentation in the Lower Estuary and Gulf of St. Lawrence, the Laurentian Trough represents a potentially important site for the accumulation of contaminants. Here, we report data on the vertical distribution of mercury in sediments and sediment pore water of the Laurentian Trough. The aim of this study is to improve our knowledge of the accumulation and possible mobility of mercury in the sediments in this coastal marine environment.

Methods

Sampling

Five undisturbed cores were collected at stations located along the longitudinal axis of the Laurentian Trough (Fig. 1) at depths of 300–500 m using an Ocean Instruments Mark II box-corer. Station 23, sampled in May 1985, and Stations 22 and 24, sampled in September 1987, are located in the landward section of the trough within the Lower St. Lawrence Estuary. Stations 12 and 13, located several hundred kilometres seaward in the Gulf of St. Lawrence, were visited in July 1988. The 35-cm-long core collected at each station was sectioned in 0.2- or 0.4-cm-thick layers near the sediment surface and then in progressively thicker layers of 1, 2, and 5 cm using the equipment for fine-scale subsampling of box-cores described by Edenborn et al. (1986). The outer 3 cm of the sediment layers that had been in contact with the core liner was not subsampled. The sediments were stored in ultra-clean prepared polyethylene bottles (Gobeil et al. 1987) and kept frozen for later chemical analysis. Aliquots for porosity determinations were also kept frozen in preweighed containers.

The core obtained at Station 23 was subsampled under a nitrogen atmosphere, and the samples were centrifuged at 4000 rpm for 20 min at 4°C to extract the pore water. The pore water sample was then taken up in a plastic syringe, filtered through a precombusted (450°C) glass fibre filter (Whatman GF/C), and stored in a sealed glass ampoule. All materials had been previously washed and rinsed according to the method described by Cossa and Courau (1990). Cossa and Fileman (1991) determined that the contamination of the samples due to the filtration procedure used in the present study is lower than the detection limit of the analytical method (2.5 pM). Moreover, it has been demonstrated that the storage of seawater in sealed glass ampoules over a 4-mo period allows the conservation of the samples without modification of the mercury concentration (Cossa and Courau 1990).

Chemical Analysis

Total dissolved mercury in pore water was measured using the analytical method of Bloom and Crecelius (1983) within 3 mo after the samples were collected. The details of our own procedure are described in Cossa and Noel (1987). Briefly, the method consists of cold-vapor atomic absorption spectrophotometry (CV-AAS) with preconcentration of mercury on a gold trap following bromination to decompose alkylmercury species (Szakacs et al. 1980), and stannous chloride reduction. For our 40-mL samples, the detection limit is 2.5 pM and the precision is better than 20% for concentrations greater than 10 pM. The accuracy of our analyses was demonstrated during the sixth

round intercalibration for trace metals in estuarine water organized by the International Council for the Exploration of the Sea (Berman and Boyko 1988). In the sample distributed by this organization, we found a mercury concentration of 32 ± 4 pM, while the consensus value was established to be 34 ± 12 pM. Dissolved iron and dissolved manganese were determined by atomic absorption spectrophotometry using an air–acetylene flame. The precision expressed as the coefficient of variation was better than 10%.

Mercury in the solid phase was measured after complete dissolution of the sediments according to the method of Toth and Ingle (1977). A known amount (~0.2 g) of freeze-dried and homogenized sample was digested in a Teflon bomb for 2 h at 90°C in a mixture of concentrated nitric (1 mL) and hydrofluoric (4 mL) acids. The solution was diluted to 50 mL while adding 3.7 g of boric acid. The mercury concentration was then determined by CV-AAS after reduction with stannous chloride. The method allows complete recovery of the mercury content (129 ± 12 ng·g^{-1}) of the National Research Council of Canada sediment standard BCSS-1 (Russel 1984). The precision, expressed as the coefficient of variation for replicate analyses ($n = 7$) of BCSS-1, is 7% and the accuracy is 2%. The detection limit is 5 ng·g^{-1}.

In order to obtain information on the association of mercury with oxide phases, the concentrations of mercury, iron, and manganese extracted from ~2 g of freeze-dried sediment with 50 mL of a hydroxylamine (0.04 M) – acetic acid (4.3 M) solution, heated at 90°C for 2 h with intermittent agitation, were also determined. The measurements were performed by atomic absorption using an air–acetylene flame for iron and manganese and by the cold-vapor technique for mercury following stannous chloride reduction. The procedure extracts manganese and iron oxides and hydroxides from the sediment as well as metals associated with these compounds (Chester and Hughes 1967; Tessier et al. 1979). The hydroxylamine – acetic acid extraction may also remove metals weakly bound to other sediment phases and metals associated with carbonates (Tessier et al. 1979).

The value of partial extraction techniques for examining the biogeochemistry of trace elements has been questioned by a number of authors (Rendell and Batley 1980; Tipping et al. 1985; Kheboian and Bauer 1987; Martin et al. 1987; Gruebel et al. 1988). The most severe criticism concerns the nonspecificity of the extractions. Also, artifacts may be introduced by post-extraction readsorption, by which an element can be remobilized from one sediment phase and readsorbed onto another during the procedure (Nirel and Morel 1990). To evaluate whether or not mercury was readsorbed on the sediment surface during the extraction procedure, five aliquots of about 2 g of the same sediment sample were spiked with 30 ng of mercury and treated with 50 mL of the hydroxylamine – acetic acid solution. The results were then compared with those obtained with aliquots of unspiked sediment extracted in the same way. This experiment failed to show any loss of mercury, within experimental error. Therefore, the hydroxlyamine – acetic acid treatment of Laurentian Trough sediments does not result in postextraction readsorption of mercury. Moreover, this treatment is not strong enough to reduce mercury, which could result in loss through volatilization. Nevertheless, partial extraction of sediment provides only qualitative information about the speciation of the metal because sample storage as well as pretreatment of the sediment samples before analysis may affect the yield of the extraction (Thomson et al. 1980; Rapin et al. 1986).

Results

The vertical distribution of total mercury in the solid phase of the sediments at all stations is shown in Fig. 2. The maximum mercury concentration decreases in the seaward direction from 520 ng·g^{-1} at the head of the Laurentian Trough to less than 100 ng·g^{-1} in the Gulf of St. Lawrence. At Stations 12 and 13 in the Gulf, the mercury concentration is nearly constant in the subsurface sediment but increases toward the sediment surface where the mercury concentration is twice that of the subsurface sediment. This trend is modified in the landward region of the Laurentian Trough where the highest mercury concentrations are found below the sediment surface at a depth that increases from Station 22 to Station 24. In this area, a constant background level is observed within the thickness of the core only at Station 22.

The concentrations of mercury in the pore water are available for the core collected at Station 23. The values ranged from the analytical detection limit (2.5 pM) to 64 pM (Fig. 3). The level of dissolved mercury was low near the sediment surface, increased to a maximum at about 5 cm depth, and then decreased with further depth. The depth below which mercury increased markedly in the pore water was about 2 cm. As is the case for mercury, both iron and manganese in the pore water were low near the sediment surface and then sharply increased with depth (Fig. 3). Dissolved manganese began to increase at about 1 cm below the sediment–water interface and dissolved iron and mercury at 2 cm.

Discussion

The concentrations of mercury in the interstitial water measured in the present study are two orders of magnitude lower than the values reported by Lindberg and Harriss (1974) for the pore waters of Mobile Bay (Alabama) and the Florida Everglades. Our values are also significantly lower than those obtained by Bothner et al. (1980) in their study of the highly contaminated Bellingham Bay (Washington). Nevertheless, the interstitial water in Laurentian Trough sediments is up to 14 times enriched in mercury relative to the overlying bottom water in which the mercury concentration, estimated from the analysis of unfiltered samples, does not exceed 4 pM (D. Cossa and C. Gobeil, unpublished data; Cossa 1990).

The depth of oxygen penetration in the sediments determined in replicate cores from Station 23 using microelectrodes varies between 0.6 and 1.5 cm (Silverberg et al. 1987). Our results for the same sampling site indicate that mercury is released from the solid phase into the pore water below this depth. The coincidental increase of mercury and iron into the pore water suggests, as already proposed by Loring (1975), that some of the mercury in the Laurentian Trough sediments is associated with iron oxides and hydroxides. We cannot exclude, however, the possibility that mercury is also associated with hydrous manganese oxides. The depths at which mercury and manganese markedly increase in the pore water are very similar, and manganese oxides are also known to be efficient scavengers for trace metals in the environment through adsorption and coprecipitation reactions (Jenne 1968; Lockwood and Chen 1973). Thus, mercury would be remobilized into the pore water when iron and manganese oxides are reduced and dissolved as a consequence of the microbial degradation of the organic matter (Froelich et al. 1979). Dissolved mercury then diffuses both upward and downward along the concentration gradients in the pore water. The precipitation

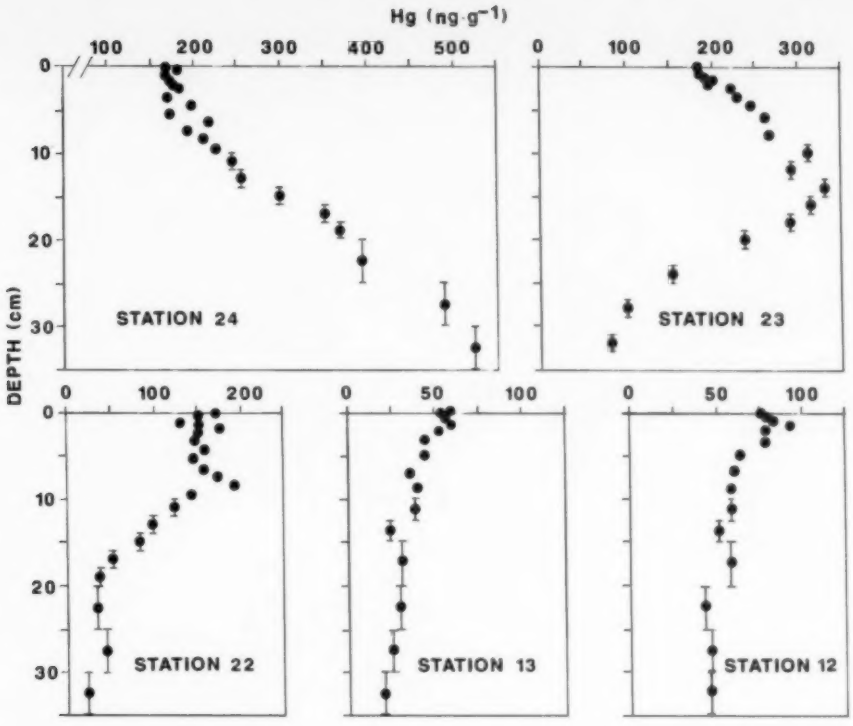


Fig. 2. Profiles of the vertical distribution of mercury concentration at the different sampling sites. Vertical bars indicate the thickness of the sediment layer analyzed.

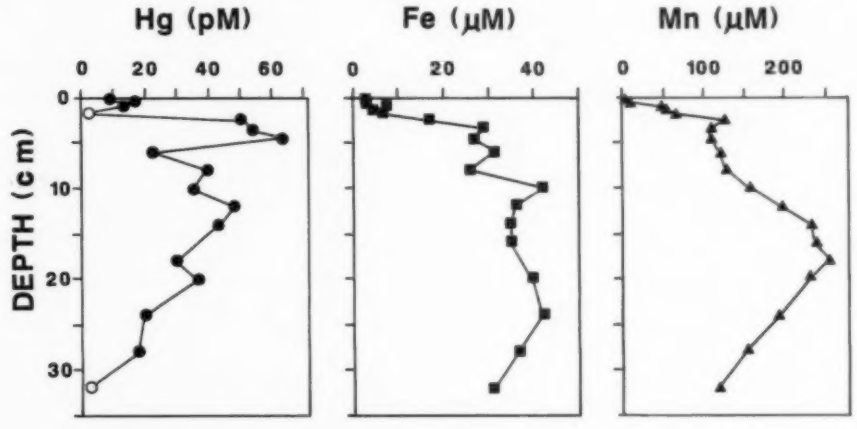


FIG. 3. Vertical profiles of dissolved mercury, iron, and manganese in the pore water at Station 23. The two values indicated by open circles are below the detection limit (2.5 pM).

of insoluble mercury sulfide and the coprecipitation of mercury with iron sulfides may provide mechanisms for the decrease in dissolved mercury below 5 cm depth in the core. On the other hand, mercury diffusing upward would adsorb onto oxidized surface sediments.

The results of the partial extraction of the sediments with the hydroxylamine – acetic acid solution (Fig. 4) represent further evidence of the role of iron and manganese oxides in the diagenesis of mercury. The simultaneous extraction of high levels of mercury, iron, and manganese in the first 1–2 cm indicate that these oxides probably do act as a sink for mercury at the sediment surface, thereby limiting the flux of mercury to the overlying bottom water. This interpretation is consistent with the existing results of in situ measurements of diffusive fluxes of mercury from sediments (Bothner et al. 1980) that demonstrate that only sediments having strongly reducing conditions at the surface can release detectable amounts of mercury to the water column.

Although the present data show that mercury is involved in

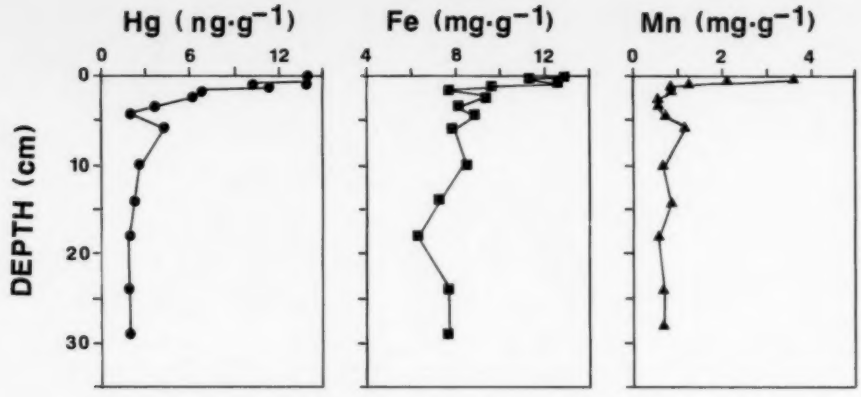


Fig. 4. Vertical profiles of mercury, iron, and manganese extracted from the sediments at Station 23 with a hydroxylamine – acetic acid solution.

diagenetic processes, the question is whether this could lead to a significant postdepositional redistribution of mercury. This can be evaluated by applying Fick's first law of diffusion to the steepest concentration gradient of mercury in the pore water (Berner 1980). The diffusive flux of mercury, J, is

$$J = -\Phi D_s(\partial C/\partial z)$$

where Φ is the porosity, D_s is the whole-sediment diffusion coefficient, and $(\partial C/\partial z)$ is the concentration gradient, z being positive downward. D_s is assumed to be $\Phi^2 D_0$ (Ullman and Aller 1982) where D_0 is the molecular diffusion coefficient for mercury in seawater. We have adopted $D_0 = 5 \times 10^{-6}$ cm^2·s^{-1} after Bothner et al. (1980) from Bellingham Bay sediments, in line with the general value of about 10^{-6} cm^2·s^{-1} for heavy metal ions and complexes (Li and Gregory 1974). The upward diffusive flux of mercury calculated from the concentration gradient that appears just below 2 cm depth then amounts to (–)0.95 ng·cm^{-2}·yr^{-1}. This flux represents only 2.6% of the present deposition rate of mercury at the sediment surface, estimated by multiplying the mercury concentration in the surficial sediment (180 ng·g^{-1}) by the sedimentation rate (~0.2 g·cm^{-2}·yr^{-1}) at Station 23 (Silverberg et al. 1986). Thus, the diagenetic redistribution of remobilized mercury within the sediment column subsequent to deposition seems insufficient to induce important variations with depth of the mercury concentration in the solid phase. At our five stations, these variations are of the order of 100–400%.

Factors such as spatial differences in vertical profiles of metals as well as biodiffusion intensity (Gobeil et al. 1987; Silverberg and Sundby 1990) could certainly affect our estimate of the mercury redistribution based on a single core. However, even if biodiffusion enhanced the transport of mercury by as much as a factor of 5 at the sampling site (Silverberg et al. 1987), this would still amount to little more than 13% of the total mercury influx. The low mobility of mercury in the sediments is also in agreement with Loring (1975), who concluded that a major proportion of the mercury content of the sediments in the Lower St. Lawrence Estuary is bound to lignin, a terrestrial component of the organic matter highly resistant to microbial degradation. It is therefore reasonable to conclude that the mercury profiles in the sediments reflect mainly the temporal changes in the rate at which mercury has been added to the Laurentian Trough, although bioturbation may affect the accuracy of the chronological record.

Changes in the input of mercury to the sediments through time can now be examined with some confidence. Constant background levels of mercury are observed at depth in the cores from Stations 12, 13, and 22. These values represent the preindustrial mercury concentration of the sediments at each site. The differences among them are not caused by analytical artefacts. Independent analyses (Gobeil 1991) also revealed marked differences in the background concentrations for other elements between these sites. They are most likely due to geographical changes in mineralogy. Thus, the sediments at Station 22 (and Stations 23 and 24, although background was not reached in the cores collected at these sites) are all derived from the crystalline rocks of the Canadian Shield. The sediments at Stations 12 and 13 in the Gulf, however, have multiples sources (i.e., the Canadian Shield to the north, the Applachian Province to the south, and the carbonate rocks of the St. Lawrence Lowlands Formation that make up the nearby Anticosti Island (Loring and Nota 1973)).

The profiles of mercury at Stations 12 and 13 near the seaward end of the Laurentian Trough in the Gulf of St. Lawrence show increasing mercury levels toward the sediment surface. The mercury concentrations in the surficial sediments at both sites are twice the values of the respective background levels, indicating that the accumulation of anthropogenic mercury is presently about the same as that of natural mercury. If we consider a sedimentation rate of 2 mm·yr^{-1} (~0.1 g·cm^{-2}·yr^{-1}) at Station 12 (Silverberg et al. 1986) and assume the same rate at Station 13, the current deposition flux of anthropogenic mercury at these stations is ~3.4 ng·cm^{-2}·yr^{-1}. This flux is not very different from the average continental depositional rate of mercury from the atmosphere, i.e. 2.5 ng·cm^{-2}·yr^{-1}, of which 50% is believed to be from anthropogenic sources (Fitzgerald 1989). A significant proportion of the anthropogenic mercury accumulating in the Laurentian Trough sediments in the Gulf of St. Lawrence is likely brought in via the atmosphere.

In the estuarine section of the Laurentian Trough, the vertical distribution profiles of mercury in the sediments are different from those observed in the Gulf. The estuarine profiles are all characterized by a subsurface maximum that appears at a depth that increases in the landward direction. This is probably related

to the release of mercury from the chor-alkali factories located in the St. Lawrence drainage basin, the only major source of mercury known to have diminished over the past two decades. Using sedimentation rates of 0.2, 0.3, and 1.6 $cm \cdot yr^{-1}$ at Stations 22, 23, and 24 (Silverberg et al. 1986), the 1970 horizon should correspond to depths of 3, 5, and 27 cm, respectively. We observed our mercury maxima at 8, 12, and more than 30 cm depth. This discrepancy is attributed to the natural variability associated with the physical mixing of the sediments by the organisms whereby the maximum levels would be deeper than otherwise predicted. According to Silverberg et al. (1986) the first 5 cm below the sediment surface in the Laurentian Trough is intensively bioturbated, although bioturbation continues to much greater depth, especially at Station 24 (Bouchard 1983; Ouellet 1983). Other studies have shown that bioturbation can affect the depth of concentration maxima in aquatic sediments (Robbins and Edgington 1975; Kramer et al. 1991). Even in the Saguenay Fjord, where bioturbation is less important than in the Laurentian Trough, anomalies in the apparent age of the mercury maximum were observed (Smith and Loring 1980).

The decrease in the seaward direction of the mercury concentration between Station 24 and Station 22 observed in this study is consistent with previous observations of the spatial distribution of mercury in the surface sediment of the estuary (Loring 1975). This early study also demonstrated that sediments with high mercury content are limited to the muddy floor of the Laurentian Trough. Using the available data, a simple model can be used to estimate the anthropogenic burden of mercury in the Lower St. Lawrency Estuary. We have divided the area below the 200-m isobath into Zones A, B, and C (Fig. 1) that we assume are represented, respectively, by our Stations 24, 23, and 22. To determine the content of anthropogenic mercury in the sediments, we have summed the total mercury in a 1-cm^2 column using the measured porosity values and a sediment density of 2.65 $g \cdot cm^{-3}$, subtracted the contribution of natural mercury, and multiplied by the surface area of each zone. The preindustrial mercury concentration in the sediments of all the zones is represented by the constant background level found at depth at Station 22, i.e. 30 $ng \cdot g^{-1}$. In order to assess the total accumulation of industrial mercury in the sediments at Station 23, where the background level was not reached, the mercury profile was extrapolated downward. For Station 24, we have assumed that total anthropogenic mercury in the sediment column is twice that calculated for the first 35 cm. Some approximation is necessary due to the high sedimentation rate at this station, the inflexion point in the mercury profile still not having been reached at 35 cm depth.

On the basis of our calculations, we estimate the total amount of anthropogenic mercury deposited in the estuary since the beginning of industrialization as 170×10^3 kg (Table 1). This value is six times higher than the amount of natural mercury deposited during the same period. Considering the spatial variability in the mercury concentration of the surficial sediments found by Loring (1975), the precision of our estimate of the total burden of anthropogenic mercury in the system could be on the order of ±50%. Some portion of the industrial mercury in the sediments has certainly been imported to this area from the Saguenay River and Fjord. The total losses of mercury into the environment from the chlor-alkali plant located on the Saguenay River have been estimated to be 234×10^3 kg (Loring and Bewers 1978). However, since 75% of the 144×10^3 kg of the mercury that has been directly discharged into the aquatic environment was buried in the Saguenay River and Fjord sediments (Loring and Bewers 1978), other sources may also have contributed to the mercury contamination of the St. Lawrence Estuary.

TABLE 1. Assessment of the total accumulation of industrial mercury deposited in the sediments below 200 m in the Laurentian Trough, Lower St. Lawrence Estuary. Zones A, B, and C are shown in Fig. 1.

Zone	Area (km^2)	Total accumulation (10^3 kg)
A	719	100
B	959	32
C	3080	38

Summary

The results of this study demonstrate that the interstitial water of the Laurentian Trough sediments is enriched in mercury relative to the overlying bottom water. Mercury is released into the pore water when iron and manganese oxides are reduced as a consequence of the microbial degradation of the organic matter. Partial extractions of the sediments with a hydroxylamine – acetic acid solution suggest that these oxides probably act as a sink for mercury at the sediment surface, thereby limiting the flux of mercury to the overlying bottom water. Although some mercury is involved in diagenetic processes, flux calculations indicate that the redistribution of remobilized mercury subsequent to deposition cannot explain more than a small proportion of the 100–400% variations with depth of the mercury concentrations in the sediments observed at all stations. The mercury profiles in the sediments therefore reflect mainly the temporal changes in the rate at which mercury has been added to the Laurentian Trough. Near the seaward end of the trough in the Gulf of St. Lawrence, the accumulation of anthropogenic mercury in the sediments is now about the same as that of natural mercury. A significant proportion of the anthropogenic mercury deposited in the Gulf sediments is likely transported via the atmosphere. In the estuarine section of the Laurentian Trough, the mercury profiles in the sediments are characterized by a pronounced subsurface maximum that can be related to the release of mercury from chlor-alkali factories located in the St. Lawrence drainage basin. Finally, the extrapolation of the data has allowed an estimate of the total burden of anthropogenic mercury in the trough sediments within the Lower St. Lawrence Estuary.

Acknowledgements

This research is a contribution to the St. Lawrence Action Plan from the Maurice Lamontagne Institute and to a Canadian–French cooperative research program between the Department of Fisheries and Oceans and the Institut Français de Recherche pour l'Exploitation de la Mer (Project No. 19). The work was supported financially by the Division of Fish Habitat Management of the Department of Fisheries and Oceans, Quebec Region. Discussions with B. Sundby and N. Silverberg and their critical comments on the manuscript were greatly appreciated. This article also benefits from the review of D. Buckley and one anonymous referee. The laboratory work was carried out with the collaboration of J. Noël and G. Paquette.

References

ASTON, S.R., D. BRUTY, R. CHESTER, AND R.C. PADGHAM. 1973. Mercury in lake sediments: a possible indicator of technological growth. Nature (Lond.) 241: 450–451.

BERMAN, S.S., AND V.J. BOYKO. 1988. ICES sixth round intercalibration for trace metals in estuarine water JMG6/TM/SW. A report for the ACMP-ICES meeting held in Copenhagen in May 1988. ICES, Copenhagen.

BERNER, R.A. 1980. Early diagenesis: a theoretical approach. Princeton University Press, Princeton, N.J. 241 p.

BLOOM, N.S., AND E.A. CRECELIUS. 1983. Determination of mercury in seawater at sub-nonogram per litre levels. Mar. Chem. 14: 49–59.

BOTHNER, M.H., R.A. JAHNKE, M.L. PETERSON, AND R. CARPENTER. 1980. Rate of mercury loss from contaminated estuarine sediments. Geochim. Cosmochim. Acta 44: 273–285.

BOUCHARD, G. 1983. Variations des paramètres biogéochimiques dans les sédiments du chenal Laurentien. M.Sc. thesis, Université du Québec à Rimouski, Rimouski, Québec. 161 p.

BRETELER, R.J., V.T. BOWEN, D.L. SCHNEIDER, AND R. HENDERSON. 1984. Sedimentological reconstruction of the recent pattern of mercury pollution in the Niagara River. Environ. Sci. Technol. 18: 404–409.

CHESTER, R., AND M.J. HUGHES. 1967. A chemical technique for the separation of ferromanganese minerals, carbonate minerals and adsorbed trace elements from pelagic sediments. Chem. Geol. 2: 249–262.

COSSA, D. 1990. Chemical contaminants in the St. Lawrence estuary and Saguenay fjord, p. 239–262. *In* M.I. El-Sabh and N. Silverberg [ed.] Oceanography of a large-scale estuarine system: the St. Lawrence Springler-Verlag, Berlin. 434 p.

COSSA, D., AND P. COURAU. 1990. An international intercomparaison exercise for total mercury in seawater. Appl. Organomet. Chem. 4: 49–54.

COSSA, D., AND C. FILEMAN. 1991. Mercury concentrations in surface waters of the English Channel: a cooperative study. Mar. Pollut. Bull. 22: 197–200.

COSSA, D., AND J. NOEL. 1987. Concentrations of mercury in near shore surface waters of the Bay of Biscay and the Gironde Estuary, Mar. Chem. 20: 389–396.

EDENBORN, H.M., A. MUCCI, N. BELZILE, J. LEBEL, N. SILVERBERG, AND B. SUNDBY. 1986. A glove box for the fine-scale subsampling of sediment box-cores. Sedimentology 33: 147–150.

FITZGERALD, W.F. 1989. Atmospheric and oceanic cycling of mercury, p. 152–183. *In* J.P. Riley, R. Chester, and R.A. Duce [ed.] Chemical oceanography. Vol. 10. Academic Press, New York, N.Y.

FROELICH, P.N., G.P. KLINKHAMMER, M.L. BENDER, N.A. LUEDTKE, G.R HEATH, C. CULLEN, P. DAUPHIN, D. HAMMOND, B. HARTMAN, AND V. MAYNARD. 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochim. Cosmochim. Acta 43: 1075–1090.

GOBEIL, C. 1991. Inventaire de la contamination des sédiments du chenal Laurentien: données sur les métaux et les éléments nutritifs. Rapp. MS Can. Sci. Halieut. Aquat. 854: iv + 63 p.

GOBEIL, C., N. SILVERBERG, B. SUNDBY, AND D. COSSA. 1987. Cadmium diagenesis in Laurentian Trough sediments. Geochim. Cosmochim. Acta 51: 589–596.

GRUEBEL, K.A., J.A. DAVIS, AND J.O. LECKIE. 1988. The feasibility of using sequential extraction techniques for arsenic and selenium in soils and sediments. Soil Sci. Soc. Am. J. 52: 390–397.

JENNE, E.A. 1968. Controls on Mn, Fe, Co, Ni, Cu, and Zn concentrations in soils and water: the significant role of hydrous Mn and Fe oxides, p. 337–387. *In* R.F. Gould [ed.] Trace inorganics in water. Adv. Chem. Ser. 73. American Chemical Society, Washington, D.C. 396 p.

KHEBOIAN, C., AND C.F. BAUER. 1987. Accuracy of selective extraction procedures for metal speciation in model aquatic sediments. Anal. Chem. 59: 1417–1423.

KLEIN, D.H., AND E.D. GOLDBERG. 1970. Mercury in the marine environment. Environ. Sci. Technol. 4: 765–768.

KRAMER, K.J.M., R. MISDORP, G. BERGER, AND R. DUIJTS. 1991. Maximum pollutant concentrations at the wrong depth: a misleading pollution history in a sediment core. Mar. Chem: 36: 183–198.

LI, Y.H., AND S. GREGORY. 1974. Diffusion of ions in sea water in deep-sea sediments. Geochim. Cosmochim. Acta 38: 703–714.

LINDBERG, S.E., AND R.C. HARRISS. 1974. Mercury – organic matter associations in estuarine sediments and interstitial water. Environ. Sci. Technol. 8: 459–462.

LOCKWOOD, R.A., AND K.J. CHEN. 1973. Adsorption of Hg(II) by hydrous manganese oxides. Environ. Sci. Technol. 7: 1028–1034.

LORING, D.H. 1975. Mercury in the sediments of the Gulf of St. Lawrence. Can. J. Earth Sci. 12: 1219–1237.

LORING, D.H., AND J.M. BEWERS. 1978. Geochemical mass balances for mercury in a Canadian fjord. Chem. Geol. 22: 309–330.

LORING, D.H., AND D.J.G. NOTA. 1973. Morphology and sediments of the Gulf of St. Lawrence. Bull. Fish. Res. Board Can. 182: 147 p.

MARTIN, J.M., P. NIREL, AND A.J. THOMAS. 1987. Sequential extraction techniques: promises and problems. Mar. Chem. 22: 313–341.

NIREL, P.M.V., AND F.M.M. MOREL. 1990. Pitfalls of sequential extractions. Water Res. 24: 1055–1056.

OUELLET, G. 1983. Étude de l'interaction des animaux benthiques avec les sédiments du chenal Laurentien. M.Sc. thesis, Université du Québec à Rimouski, Rimouski, Québec. 188 p.

RAPIN, F., A. TESSIER, P.G.C. CAMPBELL, AND R. CARIGNAN. 1986. Potential artifacts in the determination of metal partitioning in sediments by a sequential extraction procedure. Environ. Sci. Technol. 20: 836–840.

RENDELL, P.S., AND G.E. BATLEY. 1980. Adsorption as a control of metal concentrations in sediment extracts. Environ. Sci. Technol. 14: 314–318.

ROBBINS, J.A., AND D.N. EDGINGTON. 1975. Determination of recent sedimentation rates in Lake Michigan using Pb-210 and Cs-137. Geochim. Cosmochim. Acta 39: 285–304.

RUSSEL, D.S. 1984. Variable standards for use in the analysis of marine materials. Marine Analytical Standard Program No. 23025. Rep. No. 8, National Research Council of Canada, Ottawa, Ont.

SILVERBERG, N., J. BAKKER, H.M. EDENBORN, AND B. SUNDBY. 1987. Oxygen profiles and organic matter fluxes in Laurentian Trough sediments. Neth. J. Sea Res. 21: 95–105.

SILVERBERG, N., H.V. NGUYEN, G. DELIBRIAS, M. KOIDE, B. SUNDBY, Y. YOKOYAMA, AND R. CHESSELET. 1986. Radionuclide profiles, sedimentation rates, and bioturbation in modern sediments of the Laurentian Trough, Gulf of St. Lawrence. Oceanol. Acta 9: 285–290.

SILVERBERG, N., AND B. SUNDBY. 1990. Early diagenesis and sediment–water interaction in the Laurentian Trough, p. 202–238. *In* M.I. El-Sabh and N. Silverberg [ed.] Oceanography of a large-scale estuarine system: the St. Lawrence. Springer-Verlag, Berlin. 434 p.

SMITH, J.N., AND D.H. LORING. 1981. Geochronology for mercury pollution in the sediments of the Saguenay fjord, Quebec. Environ. Sci. Technol. 15: 994–951.

SZAKACS, O., A. LASZTITY, AND Z. HORVATH. 1980. Breakdown of organic mercury compounds by hydrochloric acid – permanganate or bromine monochloride solution for the determination of mercury by cold varpour atomic absorption spectrometry. Anal. Chim. Acta 121: 219–224.

TESSIER, A., P.G.C. CAMPBELL, AND M. BISSON. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51: 844–851.

THOMAS, R.L. 1970. The distribution of mercury in the sediments of Lake Ontario. Can. J. Earth Sci. 9: 636–651.

THOMSON, E.A., S.N. LUOMA, D.J. CAIN, AND C. JOHANSSON. 1980. The effect of sample storage on the extraction of Cu, Zn, Fe, Mn and organic material from oxidized estuarine sediments. Water Air Soil. Pollut. 14: 215–233.

TIPPING, E., N.B. HETHERINGTON, AND J. HILTON. 1985. Artifacts in the use of selective chemical extraction to determine distributions of metals between oxides of manganese and iron. Anal. Chem. 57: 1944–1946.

TOTH, J.R., AND J.D. INGLE. 1977. Determination of mercury in manganese nodules and crusts by cold-vapor absorption spectrometry. Anal. Chim. Acta 92: 409–412.

ULLMAN, W.J., AND R. ALLER. 1982. Diffusion ocefficients in nearshore marine sediments. Limnol. Oceanogr. 27: 552–556.

YOUNG, D.R., J.N. JOHNSON, A. SOUTAR, AND J.D. ISAACS. 1973. Mercury concentrations in dated varved marine sediments collected off southern California. Nature (Lond.) 244: 273–275.

Bias Correction of Rockfish School Cross Section Widths from Digitized Echo Sounder Data

R. Kieser, T.J. Mulligan, Laura J. Richards, and B.M. Leaman

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada

Kieser, R., T.J. Mulligan, L.J. Richards, and B.M. Leaman. 1993. Bias correction of rockfish school cross section widths from digitized echo sounder data. Can. J. Fish. Aquat. Sci. 50: 1801–1811.

The image of a fish school on an echogram represents a cross section through the actual three-dimensional school. Measurement of the size of this cross section, specifically the cross section width (or horizontal distance at a given depth), can be useful for further analysis of biomass or for species recognition. However, the measurement of fish school cross section widths from an echogram can be severely biased when the school dimensions are close to the dimensions of the transducer beam pattern. This is a particular problem for species that form small schools, especially in deep water. Using characteristics of the acoustic beam and measurements of both single fish and of school cross section widths, we developed a bias correction method. Corrected cross section widths of rockfish (*Sebastes* spp.) schools in our study area ranged from 70 m down to the dimensions measured for single fish and averaged less than 50% of the uncorrected measurements.

L'échogramme d'un banc de poissons est, de fait, une coupe de la troupe tridimensionnelle. La mesure de cette coupe, plus précisément de sa largeur (ou grandeur horizontale à une profondeur donnée), peut être utile à l'analyse de la biomasse ou à la détermination de l'espèce. Toutefois, cette mesure risque d'être entachée d'une erreur systématique grave lorsque la taille du banc approche des dimensions du faisceau du transducteur. Cela fait particulièrement problème lorsque l'espèce forme de petits bancs, notamment en eau profonde. À partir des caractéristiques du faisceau acoustique et des mesures de la largeur d'un seul poisson et de bancs de poissons, nous avons mis au point une méthode de correction de l'erreur systématique. La largeur corrigée des bancs de sébastes (*Sebastes* spp.) de la région que nous avons étudiée allait de 70 m à la dimension mesurée d'un seul poisson. En moyenne, elle était inférieure à la moitié de la largeur brute.

Received February 28, 1991
Accepted February 26, 1993
(JA923)

Reçu le 28 février 1991
Accepté le 26 février 1993

Off the west coast of North America, hydroacoustic surveys contribute routinely to assessments of several major species, including Pacific hake (*Merluccius productus*), Pacific herring (*Clupea pallasi*), and walleye pollock (*Theragra chalcogramma*) (Megrey 1991; Sullivan 1991). These species make good hydroacoustic subjects because they can be surveyed at times of year when they form large midwater schools with only minor contamination from other fish species. By echo sounding along a series of transect lines and measuring the returned echo intensity with a calibrated hydroacoustic system, an estimate of fish biomass can be obtained (Forbes and Nakken 1971; Burczynski 1982). This experience has led researchers to attempt hydroacoustic surveys for other economically important species that are less amenable to acoustic methods.

In this paper, we describe some of the problems encountered when surveying species that form small schools, especially in deep water. We present a simple procedure to define a single school from digitized acoustic data and extend the method of Smith (1970) to measure its cross section width, i.e., its length along the direction of the transect. (This use of the term "cross section" refers to the shape of the school as observed on an echogram and should not be confused with backscattering cross section, a measure of the target's acoustic reflectivity.) Although we do not estimate school biomass here, measurement of school cross section widths forms one component of biomass estimation. Furthermore, school cross section measurements are integral to studies of fish behaviour and species recognition. We illustrate our methods with data from rockfish (*Sebastes* spp.) and demonstrate that some school cross section widths can be as narrow as a single fish, while others have a cross section width as large as 70 m.

The methods we present are particularly important for small schools because the apparent cross section width can be much larger than the actual width. The echoes observed from a school or single fish represent the integral of the product of the directional sensitivity of the acoustic system (the beam pattern) and the stochastic backscattering from the target (Clay and Medwin 1977; Mitson 1983). This well-understood process, called convolution, has been treated in detail for the case of single fish (Craig and Forbes 1969; Clay and Medwin 1977; Clay and Heist 1984). Although these and other authors normally confine their attention to the echo amplitude and its transformation as a result of the convolution, the spatial dimensions of the targets are also affected. Target measurements obtained from the acoustic image (i.e., the target's appearance on the echogram) are biased unless they are corrected for the acoustic beam width. This bias can be negligible for schools with cross section widths much larger than the beam width. However, a relatively large bias was obtained for the narrow, deep rockfish schools in our study.

The appearance of a single fish as an "inverted v" (IV) on the echogram is a familiar result of the convolution process (Mitson 1983). These IVs do not resemble the target shape and become

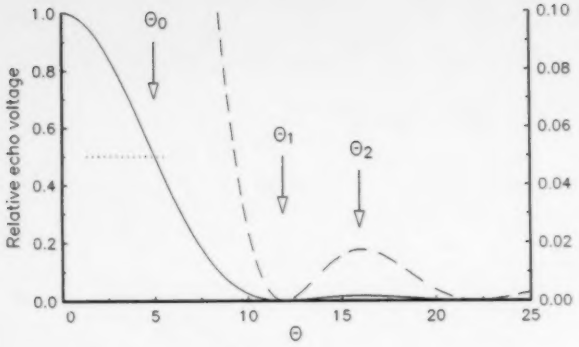


FIG. 1. Typical beam pattern for a cylindrical piston transducer versus the beam angle θ. The broken line represents the solid line times a factor of 10. The left vertical axis gives echo voltage (one-way power) and refers to the solid line, while the right axis refers to the broken line. The half power point on the beam pattern is shown by the intersection of the dotted line with the solid line. Values of the half power angle, θ_0, the first minimum, θ_1, and the maximum of the first side lobe, θ_2, are shown.

wider as depth below the transducer increases. This phenomenon arises because single fish represent a point source target and the acoustic beam has a conical cross section. The bias correction method we describe is related to the "duration in beam" studies for single fish (Nunnallee 1980; Crittenden et al. 1988; Crittenden and Thomas 1992). These studies examine the IV chord lengths as a function of distance from the transducer and attempt to describe how long the fish remains in the beam, or alternatively, to describe the mean beam width for the size distribution of fish. An idealized model of a single fish in an acoustic beam shows that the largest chord length for the IV image is given by $2R \sin \theta$ where R is the distance from the transducer and θ is the beam angle. Because θ depends on target strength, which is not constant for all of the fish in a given population, the beam angle will also vary. Duration in beam methods describe procedures for calculating the "effective beam angle" (expectation value of the beam angle) for the observed fish size distribution.

We use both single fish and school section widths to estimate the effective beam angle (which we term the critical angle) for the rockfish we observed. We estimate this critical angle by using a classification procedure, similar in intent to standard linear discriminant analysis. The purpose of this classification procedure is to estimate the slope of the straight line that best separates the single fish and school measurements as a function of R. Once the critical angle has been determined, the bias can be subtracted from the measured school cross section widths.

Materials and Methods

Digital Data and the Acoustic Model

Before presenting the model relating cross section width (C), critical angle (θ_c), and distance to the transducer (R), we examine the digitized acoustic data and describe how cross section widths can be estimated from such data. Consider acoustic data collected from a vessel moving along a survey transect. Let h be the horizontal distance travelled between consecutive transmissions (pings) from the acoustic system and v be the depth increment between digital samples of the echo signal. We can represent the amplitude of the acoustic echoes from a portion of a transect as a matrix, $\mathbf{Z}$, with a one-to-one correspondence between the row index, i, and the column index, j, of matrix element z_{ij} and the associated location on the echogram. Thus, $\mathbf{Z}$ is a discrete numerical representation of the echogram and can be considered to represent echo amplitudes from a vertical plane in the water along the transect. Acoustic measurements represent a many-to-one transformation (the convolution process referred to in the introduction) between the actual three-dimensional position of targets in the water and the position of the resultant point on the two-dimensional echogram. Let x_0 and y_0 be the horizontal and vertical offsets from a reference point, such as the sea surface at the start of the transect. Then

$$x_j = x_0 + h \cdot j \tag{1a}$$

$$y_i = y_0 + v \cdot i \tag{1b}$$

describe the location. Typically, the reference point lies outside the measured $\mathbf{Z}$-space, i.e., our measurements are far from the start of the survey transect. We use (x_j, y_i) coordinates to refer to positions in a reconstructed acoustic image, while the corresponding echo amplitudes, measured in volts, are given by z_{ij}.

Given (1), the Euclidian distance between two arbitrary points with spatial coordinates (x_j, y_i) and (x_l, y_k), corresponding to matrix coordinates (i, j) and (k, l), is

$$\begin{aligned} d &= [(x_j - x_l)^2 + (y_i - y_k)^2]^{1/2} \\ &= [h^2 (j-l)^2 + v^2 (i-k)^2]^{1/2}. \end{aligned} \tag{2a}$$

Due to the discrete nature of $\mathbf{Z}$, the spatial boundary of a target typically lies between adjacent z_{ij} elements. Thus, (2a) underestimates the target cross section width. For example, consider a continuous horizontal target first encountered at spatial coordinates (x_j, y_i). The target must have exceeded the recording threshold between digital samplings at (x_{j-1}, y_i) and (x_j, y_i). If the probability of exceeding the threshold is uniform over the interval x_{j-1} to x_j, then the expected location for the threshold value is at the midpoint of this interval, $x_{j-1} + h/2$. Since the horizontal target will have two end points, two increments of $h/2$ should be added to the observed cross section width. Analogous arguments apply to vertical boundaries. Thus,

$$\begin{aligned} \hat{d} = [h^2 (|j-l| + 1 - \delta_{jl})^2 \\ + v^2 (|i-k| + 1 - \delta_{ik})^2]^{1/2} \end{aligned} \tag{2b}$$

is the estimated Euclidian distance between two opposing target boundaries, corresponding to matrix coordinates (i, j) and (k, l). In (2b), δ_{ij} is the Kronecker delta function defined as

$$\begin{aligned} \delta_{ij} &= 1, \text{ if } i = j \\ &= 0, \text{ if } i \neq j \end{aligned}$$

and is used to allow this equation to assume the correct form when we are concerned with only horizontal (or vertical) dimensions. The terms (+1) in (2b) will only be important when $|j-l|$ or $|i-k|$ (the school dimensions, measured in $\mathbf{Z}$ column or row intervals) is small.

The acoustic model that relates the observed and actual school cross section width is based on the transducer beam pattern (Fig. 1). Given a point target of fixed strength and range from

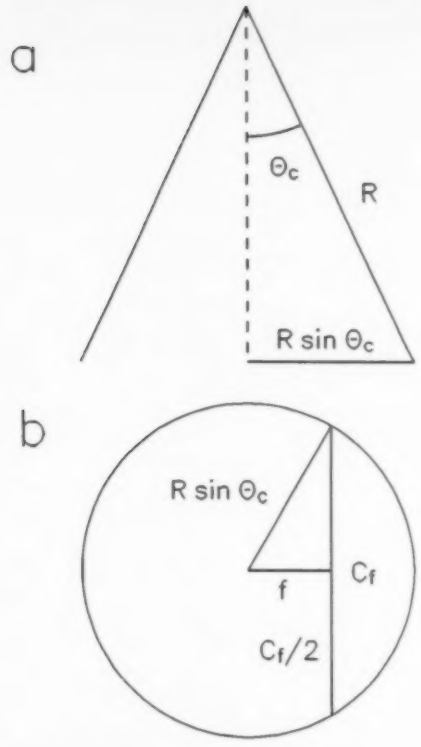


FIG. 2. Beam geometry. (a) Vertical section of the acoustic beam showing the relationship between the beam radius (horizontal line) and the range, R. (b) Horizontal section of the acoustic beam at range R. The chord length, C_f, is obtained when a single fish passes through the beam with a horizontal offset of f from the beam axis.

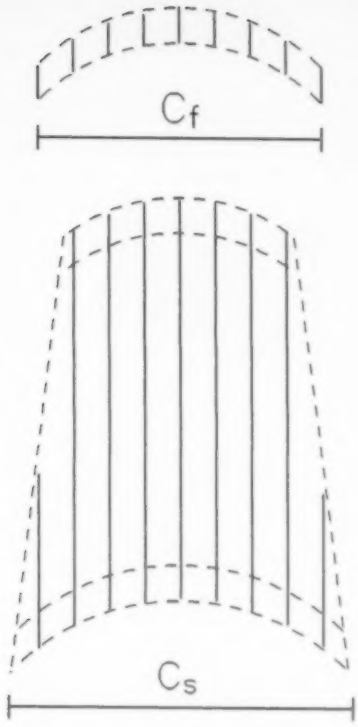


FIG. 3. Hypothetical acoustic images for a single fish (top image), which is a point source target, and a vertical line school, which is an extended source target. The school image represents the superposition of many individual point source targets.

the transducer, the measured echo amplitude depends only on the target's angular location in the beam. For a cylindrically symmetric beam pattern, the measured signal depends on θ, the angle between the target and the acoustic axis of the beam. Figure 1 shows three important angles that characterize the beam: θ_0, the half power point; θ_1, the first minimum of the beam pattern; and θ_2, the maximum of the first side lobe. As the target moves outward from the beam axis, its echo amplitude follows the beam pattern curve. Beyond some point, which we call the critical angle θ_c, the amplitude will be so low that the target can no longer be detected, i.e., it will fall below the detection threshold. Typically, this threshold for the acoustic system is chosen so that θ_c for the targets of interest corresponds to an amplitude greater than the amplitude at θ_2. This ensures that the targets will be observed only in the main lobe of the beam, not in the side lobes. The value of θ_c will be larger for intense targets, such as dense schools, than for weak targets, such as a single fish. Normally, one would expect $\theta_0 < \theta_c < \theta_1$ for single fish, while for a very dense school or the bottom, θ_c approaches θ_1.

The cross section width for a single fish can also be expressed in terms of these quantities. Assuming a vertically downward looking transducer in a moving vessel, the cross section width for a single fish (Fig. 2) will be given by the chord length (C_f) of the target's trajectory through the beam:

$$(3)\quad C_f/2 = [\,(R \sin \theta_c)^2 - f^2\,]^{1/2}.$$

In (3), R is the maximum range from the transducer, i.e., when the target is furthest from the centre of the beam, and f is the horizontal offset between the beam axis and the target. From Fig. 2 and (3), it is evident that the condition

$$(4)\quad 0 \le C_f \le 2R \sin \theta_c$$

will hold for single fish targets.

If we were to substitute observed values of R and θ_c into (4), we would find that the dimensions of the reconstructed IV are much larger than the actual targets. Similarly, the IV shape is not representative of the target's shape. While we recognize the IV as a distortion of the single fish image, we generally ignore the same distortion process acting on fish schools. The single fish target represents a point source to the acoustic system. Because fish schools can be regarded as a superposition of point source targets, IV features can be extended to fish schools. (When we use the term school in this paper, we mean an acoustically unresolved, multiple fish aggregation.)

Consider a very narrow, vertical school that is large in the y-dimension, but essentially a point source in the x-dimension (Fig. 3). This "vertical line" school represents a uniform column of fish, with one fish positioned precisely above another. By analogy with (4), a vertical line school passing through the centre of the beam has a cross section width (C_s) given by $2R \sin \theta_c$. In general, the cross section width of a school must be at least as

large as that of a vertical line school and therefore satisfies the relation

$$(5)\quad C_s \geq 2\ R \sin \theta_c$$

where the equality is valid only for a vertical line school. If schools do not pass through the centre of the acoustic beam, the measured cross section width can fall below the limit defined by (5). These schools can usually be identified by their smaller echo amplitude. In our analysis, we compare school and single fish amplitudes to develop criteria that help exclude off-axis schools.

Provided that we have an estimate for θ_c (see below for the estimation procedure), we can remove the bias from the observed values of C_s. Mathematically, the corrected school cross section width (C_s^*) is estimated by

$$(6)\quad C_s^* = C_s - 2R \sin \hat{\theta}_c .$$

Negative C_s^* estimates should be interpreted as vertical line cross section widths. Note that the bias increases with increasing R. Thus, deep, narrow schools will have a larger relative bias then the same size school at shallow depths.

The estimation of θ_c was accomplished by performing a constrained classification analysis on the combined C_f and C_s data. This classification algorithm determines the straight line that passes through the origin in the (C, R)-space and best separates the observed C_f values from the C_s values. We defined a misclassification score (S) to be the number of observations that occurred on the wrong side of the line $C = 2R \sin \theta_c$ for both single fish and schools. Here, C and R are part of the observed data, while θ_c was varied over a range of values. Thus, S is given by

$$(7a)\quad S = \sum_{s=1}^{n_s} m_s + \sum_{f=1}^{n_f} m_f$$

where $s = 1, \ldots, n_s$ indexes school and $f = 1, \ldots, n_f$ indexes single fish observations. In (7a), each school is given a misclassification score (m_s) where

$$(7b)\quad m_s = 1 \text{ if } C_s < 2R \sin \theta_c$$
$$= 0 \text{ if } C_s \geq 2R \sin \theta_c$$

and similarly for single fish, m_f is given by

$$(7c)\quad m_f = 1 \text{ if } C_f > 2R \sin \theta_c$$
$$= 0 \text{ if } C_f \leq 2R \sin \theta_c .$$

Therefore, S represents the total number of observed cross section widths that were misclassified as a function of θ_c. To estimate θ_c, 100 test values were equally spaced over the range $5.7° \leq \theta_c \leq 8.5°$ and the estimate, $\hat{\theta}_c$, was assigned as the value giving the minimum S. This analytic procedure is similar in intent to linear discriminant anlaysis (Duda and Hart 1973); however, no assumptions about the distributions of C_f and C_s are required. The form of the separation line ($C = 2R \sin \theta_c$) was chosen to satisfy (3)–(5) and θ_c is interpreted as the critical angle.

Data Collection

Acoustic data were collected in Brooks Bay (50°14′N, 128°15′W) on the west coast of Vancouver Island, Canada, during a cruise on the R/V *John P. Tully* in March 1989. Complete cruise details are given in Matthews et al. (1989) and an analysis of the echo integration data is reported in Richards et al. (1991). The data, collected between 03:00 and 11:10 on March 17, 1989, represent four repetitions of a four-transect sequence, with transects being roughly perpendicular to the 200-m depth contour. Data were digitized over a range of 40–200 m. Bottom depths varied from approximately 50 m on the continental shelf to over 275 m on the continental slope.

A chartered fishing vessel deployed sunken gill nets in the same area from which the echo soundings were obtained (Matthews et al. 1989). Rockfish dominanted all catches with as many as nine rockfish species caught in a single set. A total of 13 species of rockfish were recorded during the cruise. We assume that all acoustic measurements pertain to rockfish, while recognizing that a school may be composed of several species. These fish exhibited marked diel behaviour (Richards et al. 1991). Nocturnally, they appeared off bottom in loose aggregations; diurnally, they formed compact schools, sometimes extending to the bottom. Single fish measurements were obtained predominantly from nocturnal sequences, while measurements of fish schools were from diurnal sequences in similar locations.

The acoustic equipment (Kieser et al. 1987) included a 38-kHz ceramic transducer, an echo sounder with a time-varied gain of $20 \log(R) + 2\alpha R$ (the quantity α is the absorption coefficient for the acoustic signal in water), and a digital audio tape recorder. The echo envelope voltage was digitized at 0.1-m depth intervals. The transducer was mounted in a torpedo-shaped body and towed behind the vessel at 8 knots (14.8 km/h) at a depth of ~25 m. The transducer emitted a pulse of 0.6-ms duration at 50 pulses/min. Hence, the quantities $h = 4.9$ m, $v = 0.1$ m, and $y_0 = 24.9$ m characterize the spatial properties of our data.

Our transducer is a rectangular rather than circular piston. Its measured beam pattern gave $\theta_0 = 4.0°$ and $\theta_1 = 9.4°$ for the narrow part of the beam, which was aligned along the direction of travel. Since C is determined by the fore-aft dimension of the beam and we are interested only in the maximum cross section width for a single fish, as in (4), or the minimum cross section width for a school, as in (5), the rectangular shape of the transducer does not affect our analysis.

With the time-varied gain setting used to record the data, the intensity of a given point target decreases with increasing range. To eliminate this range dependency, the amplitudes z_{ij} can be scaled by R, assuming a reference range of R_0 (Forbes and Nakken 1971; Burczynski 1982). A new matrix, $\boldsymbol{W}$, with elements w_{ij} is obtained from the relationship

$$(8)\quad w_{ij} = \frac{z_{ij} R}{R_0} .$$

We chose $R_0 = 200$ m, corresponding to the maximum range digitized. Equation (8) simulates a time-varied gain of $40 \log(R) + 2\alpha R$ that is generally used for single fish targets. A time-varied gain of $20 \log(R) + 2\alpha R$ is routinely used for single fish or schools when estimating fish densities. However, our interest is estimating boundaries of fish aggregations, a situation in which ultimately only a single fish will remain in the beam. Therefore (8) gives the appropriate transformation for measuring both single fish and school cross section widths for comparison with the relations (4) and (5).

Measurements on single fish were obtained from sections of the survey data in which fish appeared dispersed on the echogram. Initially, data matrices $\boldsymbol{W}$ were selected from areas along our transects, and all matrix elements coresponding to IVs were examined. Since fish were concentrated at certain ranges, this procedure led to a skewed distribution of sample size with range. To achieve a more uniform distribution, the appropriate rows of matrices from additional areas within the survey were selected. The final sample depth distribution was approximately uniform between ranges of 50 and 200 m.

Because echoes can come from both fish and other sources, typical acoustic selection criteria were used to choose echoes from single fish. We adopted the following five criteria: First, the data were recorded with a threshold of 0.1 V to eliminate background noise, so that $z_{ij} \geq 0.1$. Thus, at $R = R_0$ in (8), the minimum value of w_{ij} will be 0.1, while for $R < R_0$, it will be less than 0.1. Second, there must be at least one value of $w_{ij} \geq 0.2$ within the set of elements representing an IV for data to be included in the analysis. This ensured that single fish echoes would be at least twice the value of the recording threshold. If V_f is the maximum value of w_{ij} for the set of elements representing an IV, we required $V_f \geq 0.2$. Third, we excluded any IV that contained values of $w_{ij} < 0.1$ for an element w_{ij} not on a boundary, i.e., no "holes" were allowed within an IV. If $w_{ij} < 0.1$, then either condition ($w_{i,j-1} < 0.1$) or ($w_{i,j+1} < 0.1$) must be true for any coordinate pair (i, j) within the IV and adjacent to the boundary. Fourth, we chose IVs that were insonified at least twice, so that from (2b), $|j - l| + 1 \geq 2$, for column indices (j,l) within the IV. Fifth, we excluded IVs that overlapped or were adjacent to the bottom. A total of 129 IVs met our selection criteria from the matrices examined.

The measurements of C_f for each IV were obtained directly from $\boldsymbol{W}$. We counted the appropriate number of columns of $\boldsymbol{W}$ within the IV and applied the scale factor h. To illustrate, consider the minimum rectangle that completely encloses those matrix elements representing one IV. Let (i,j) be the coordinates of the upper left-hand corner and (k,l) be the coordinates of the lower right-hand corner of the rectangle. Then,

$$(9) \quad C_f = h(|l - j| + 1)$$

estimates the single fish cross section width by application of (2b).

In addition to cross section width measurements, V_f for each IV was recorded (Fig. 4). As expected, the number of observed echoes decreases rapidly as V_f increases (Clay and Medwin 1977; Clay 1983). If all of these fish were of identical target strength, the largest V_f would result from those fish that pass closest to the beam axis. Since fish differ in target strength, values of V_f differ correspondingly for fish in the centre of the beam. From Fig. 4, we selected 1.5 as a representative amplitude for fish that passed near the beam axis. We use the value 1.5 to reject schools that were only grazed by the beam. This selection is subjective. If we knew the fish size distribution, we could make a statistical selection of this representative amplitude. The observed size distribution from the associated sunken gillnet catches (Matthews et al. 1989) is most likely not representative of the true distribution due to gear selectivity and was not used in the selection process.

Criteria for the selection of rockfish schools were also established. Cross section widths were measured at 1-m range increments (every tenth row of $\boldsymbol{W}$) throughout each school. To

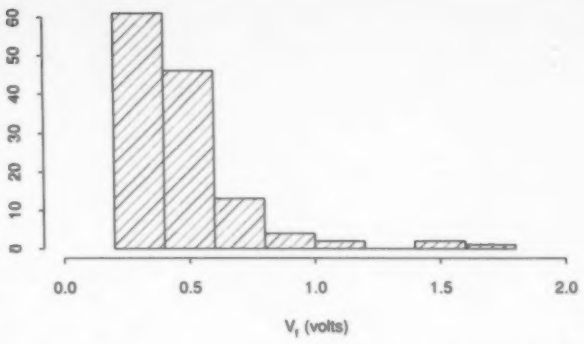


FIG. 4. Histogram of the maximum echo amplitude, V_f, observed within an individual fish echogram image.

reduce the incidence of measurements from sources other than rockfish schools, similar criteria to those described for single fish were used. First, we examined all schools whose echogram images showed well-defined boundaries and ignored loose aggregations. Second, we allowed holes to be present within the school boundary, provided that the hole extended over only one column of $\boldsymbol{W}$, i.e., if $w_{ij} < 0.1$, then both conditions ($w_{i,j-1} > 0.1$) and ($w_{i,j+1} > 0.1$) must be true for row i to be included. Holes were allowed within a school because the echo amplitude variation, both with depth and between adjacent pings, is greater for schools than for single fish. This variation is due to interference of the echo amplitudes from adjacent fish. Third, we adopted a school selection criterion that requires $w_{ij} \geq 1.5$ for at least one element w_{ij} for each row i. Thus, for schools, $V_s \geq 1.5$ where, by analogy with V_f, V_s is the maximum amplitude within the row. This criterion was chosen to minimize echoes from single fish, from schools composed of fish other than rockfish, or from schools not passing through the centre of the acoustic beam. Finally, no schools were selected that adjoined the bottom, so that the determination of the school boundaries was straightforward. In total, 633 rows from 76 schools were examined. Application of these criteria resulted in 67 sets of cross section range amplitude measurements. School cross section widths (C_s) were defined analogously to those for single fish. Thus,

$$(10) \quad C_s = h(|l - j| + 1)$$

estimates C_s where l and j represent the column indices of the school boundary for a given row of $\boldsymbol{W}$.

Results

Most of our IVs undoubtedly represented fish that did not pass through the centre of the beam. The symmetric IV in Fig. 3 will be observed only if the acoustic axis is stable and vertical and if the fish is stationary. Many IVs in our data were asymmetric, indicating that the acoustic axis deviated from vertical or that the fish moved during insonification. Our transducer was not perfectly stable, having roll, pitch, and yaw motions.

The 129 observations of C_f and 67 observations of C_s are plotted versus R in Fig. 5. The dependence of C_f on R is a prominent feature of this plot. The region of overlap in the distributions of C_f and C_s in (C,R)-space is quite limited. A straight line passing through the origin does indeed provide reasonable

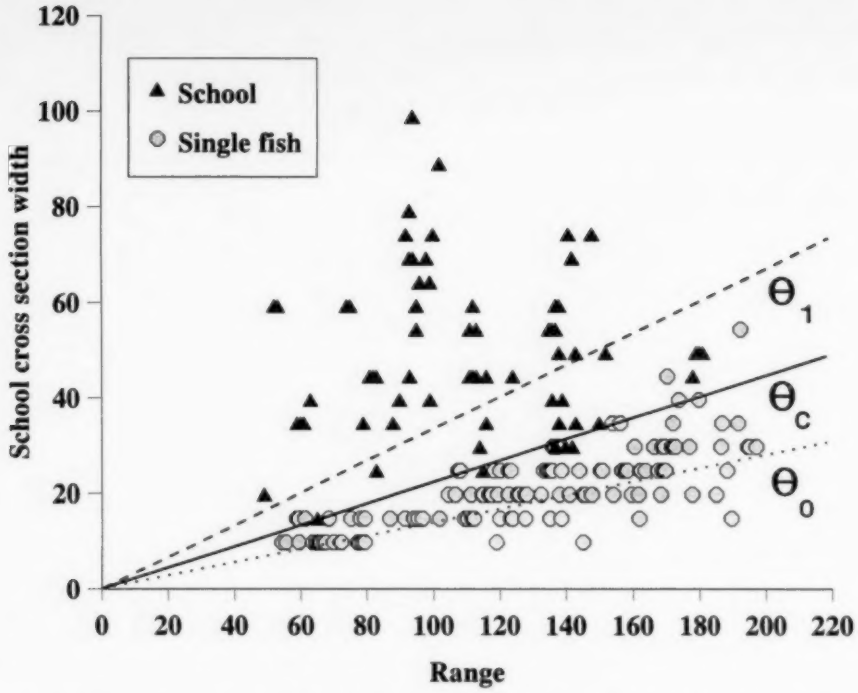


FIG. 5. Cross section widths (m) of single fish and schools versus range (m) from the transducer. The lines show the transducer beam width as a function of range for three values of the beam angle, θ. The line corresponding to the critical angle, $\hat{\theta}_c$, uses the estimate for θ_c with the lowest misclassification score.

separation between these two distributions, as predicted by (4) and (5).

To verify the agreement of school and single fish selection criteria, we examined the C_s versus R relationship for several values of V_s (Fig. 6). For values of $V_s < 1.5$ (Fig. 6a, 6b, and 6c), there is a ragged horizontal lower bound for the distribution of C_s versus R. For $V_s \geq 1.5$ (Fig. 6d), the lower bound suggests a line passing through the origin with a slope intermediate between θ_0 and θ_1, as would be expected from (5).

After establishing that the selection criteria for C_f and C_s give reasonable results, we analyzed these data sets simultaneously to determine an optimum value for θ_c. The data for this analysis consisted of values of C_f, C_s, and R. If rockfish sometimes form vertical line schools, then it should be possible to estimate θ_c by finding the value of θ that best separates C_s values from C_f values. We used the scoring algorithm to estimate θ_c. A minimum of 10 misclassifications (5 values of C_s and 5 of C_f) occurred at 6.56° (Fig. 7); therefore, $\hat{\theta}_c = 6.56°$. However, the misclassification score, S, is essentially flat in the range 6.5°–7.75°. When we plot the line $C = 2R \sin \hat{\theta}_c$, the misclassified points are readily identified (Fig. 5). With 129 (single fish) + 67 (schools) = 196 observed cross section widths and 10 misclassifications, the misclassification rate was approximately 5%.

To determine a confidence interval for $\hat{\theta}_c$, we performed two sets of bootstrap calculations (Efron 1982). Typically, there were several values of θ for a bootstrap sample that would give the same misclassification score (Fig. 7). To estimate the range of θ for which the misclassification score was minimum, two groups of 1000 bootstrap replications were performed. The first set of 1000 bootstrap subsample replications recorded $\hat{\theta}_c = \theta_s$ where θ_s was the smallest value of θ that gave the minimum value of S, while the second set of 1000 recorded $\hat{\theta}_c = \theta_L$ where θ_L was the largest value of θ that gave the minimum value of S. Figure 8 shows the histogram of these $\hat{\theta}_c$ values from the 2000 estimations. Although the bootstrap 95% confidence interval (5.94°–8.08°) is both wide and asymmetric, the overall distribution is highly peaked close to the point estimate at $\hat{\theta}_c = 6.56°$. The ragged appearance of this histogram results from the discrete nature of both the cross section width data and the scoring algorithm and from the small number of schools and single fish that influence the estimation of θ_c, i.e., those cross section widths that lie near the θ_c line in Fig. 5. With only 10 misclassifications at $\hat{\theta}_c$, many bootstrap samples are likely to include an even smaller number of these influential cross section widths.

The C_s^* values can be easily related to their original C_s value by comparing plots of these cross section widths versus R (Fig. 9). The 95% confidence interval for C_s^* can be obtained by substituting the appropriate confidence interval end points of $\hat{\theta}_c$ into (6). Figure 9a demonstrates that both the bias correction and the confidence interval increase with R, while in Fig. 9b the asymmetry of the confidence interval is readily apparent and all confidence intervals are shown to include at least some nonnegative portion. The impression about the size distribution of the rockfish school cross section widths in this study is dramatically affected by the removal of the bias (Fig. 10). The mean school size is 49.5 m for uncorrected schools, while for

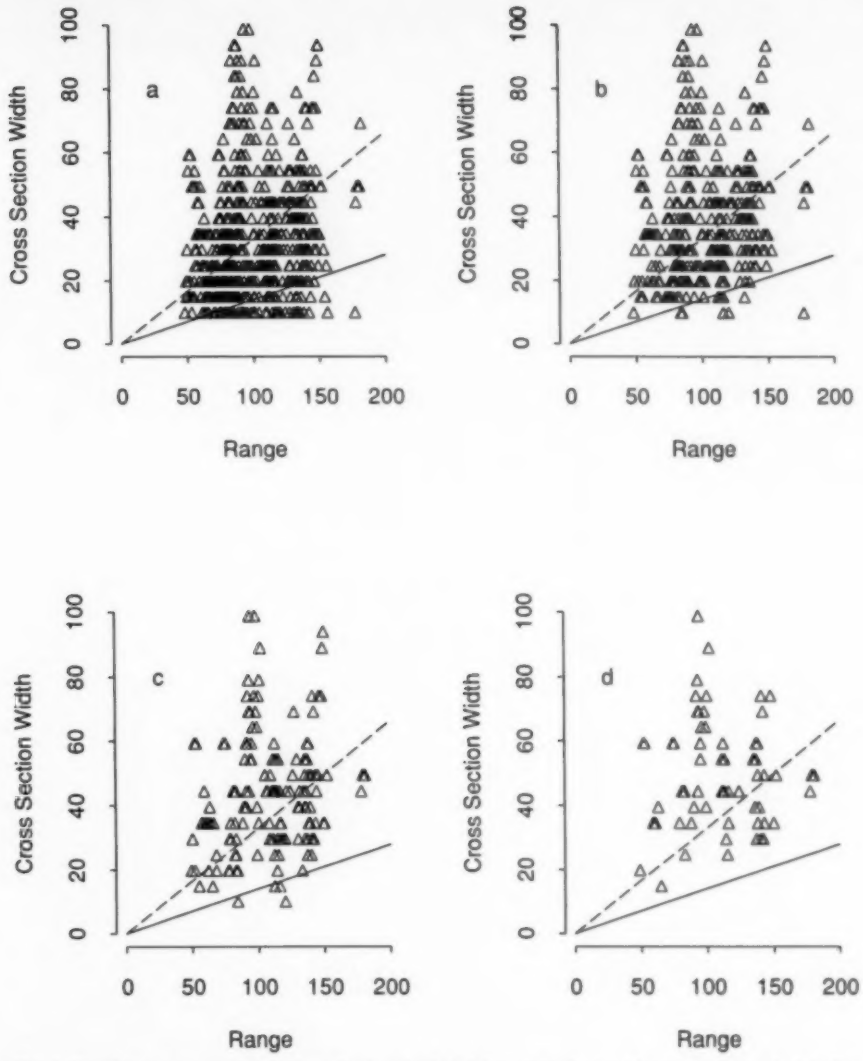


FIG. 6. Consistency among criteria for selecting single fish images and school images composed of similar fish is demonstrated. The solid and broken lines represent the beam width for θ_0 and θ_1, respectively. The panels show plots of the school cross section width (m) versus range (m) for the following: (a) $V_s \geq 0.1$, (b) $V_s \geq 0.5$, (c) $V_s \geq 1.0$, and (d) $V_s \geq 1.5$ V.

bias-corrected schools it is 24.2 m. The shape of the histogram of C_s^* values is much more asymmetric than that of C_s values. In addition, the standard deviations for each of these two distributions do not encompass the mean value of the other distribution. The narrowest parts of these schools must result from vertical line placement of the fish. It is important to note that the acoustic data represent only a two-dimensional cross section width from a three-dimensional school. Therefore, we have no information on the distribution of fish along the direction perpendicular to the vessel movement.

Concluding Remarks

As the amount of information required to manage fisheries continues to grow, measurements of individual school properties, such as cross section widths, will become more important. Our own interest in these school properties grew out of the need to estimate rockfish biomass in a mixed-species area. The large number of co-occurring rockfish species on the Pacific coast presents formidable challenges to fisheries management. The highly similar morphology, hence similar acoustic signature, of these congeners provides little discriminatory power for species recognition. However, behavioural differences and school characteristics are potential discriminators for these mixed-species locations (Richards et al. 1991). Other studies suggest that rockfish aggregations may indeed harbour several species, which agrees with species composition from trawl sampling in these areas (Leaman et al. 1990). These studies show that differences between species may be subtle or expressed only under particular conditions of illumination and current. For example, as night

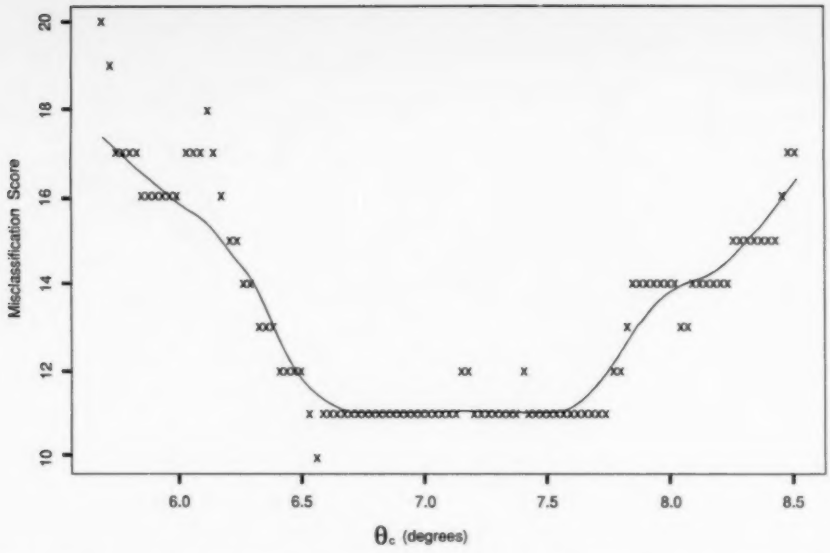


FIG. 7. Misclassification score from the classification analysis versus the value of θ_c. The line is obtained from a smoothing algorithm.

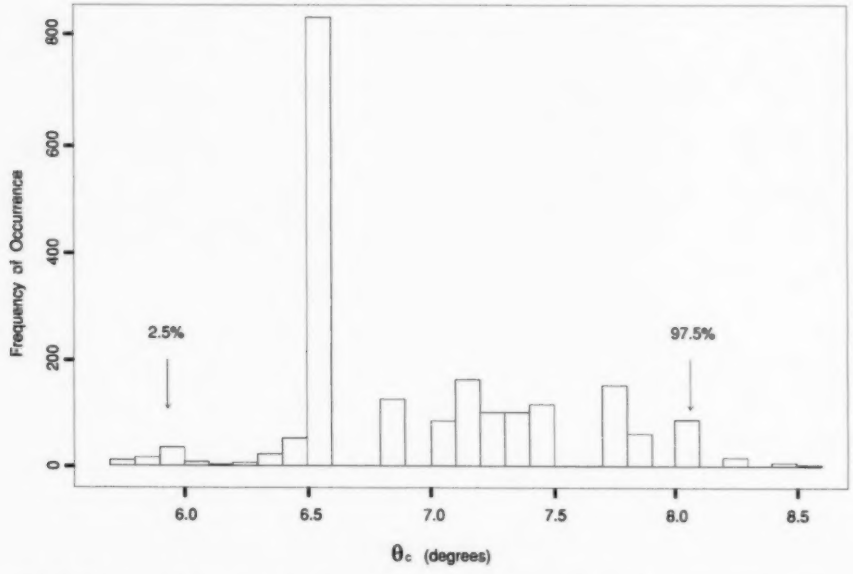


FIG. 8. Histogram of critical angle estimates, $\hat{\theta}_c$, from 2000 bootstrap calculations. The 95% confidence interval end points are shown by the arrows. Almost half of the total estimates are represented by the single large histogram bar that spans the point estimate for $\hat{\theta}_c$ at 6.56°.

approaches, these schools disaggregate and expose distinct subaggregations.

Acoustic measurement of deepwater fish is becoming more common throughout the world, and in some cases, even greater demands may be made of acoustic data analysis. For example, orange roughy (*Hoplostethus atlanticus*) fisheries off New Zealand are conducted at depths of 800–1000 m (Clark and Tracey 1991, 1992). Echograms of this species often exhibit narrow schools at depths over three times as great as those at which we have demonstrated a serious bias in the measurement of school dimensions. Clark and Tracey noted the difficulty of consistently recognizing echogram traces of orange roughy at these great depths, particularly when other species are present. Bias correction may provide additional information to separate the acoustic signatures of these species.

Our bias correction procedure is a refinement of the method

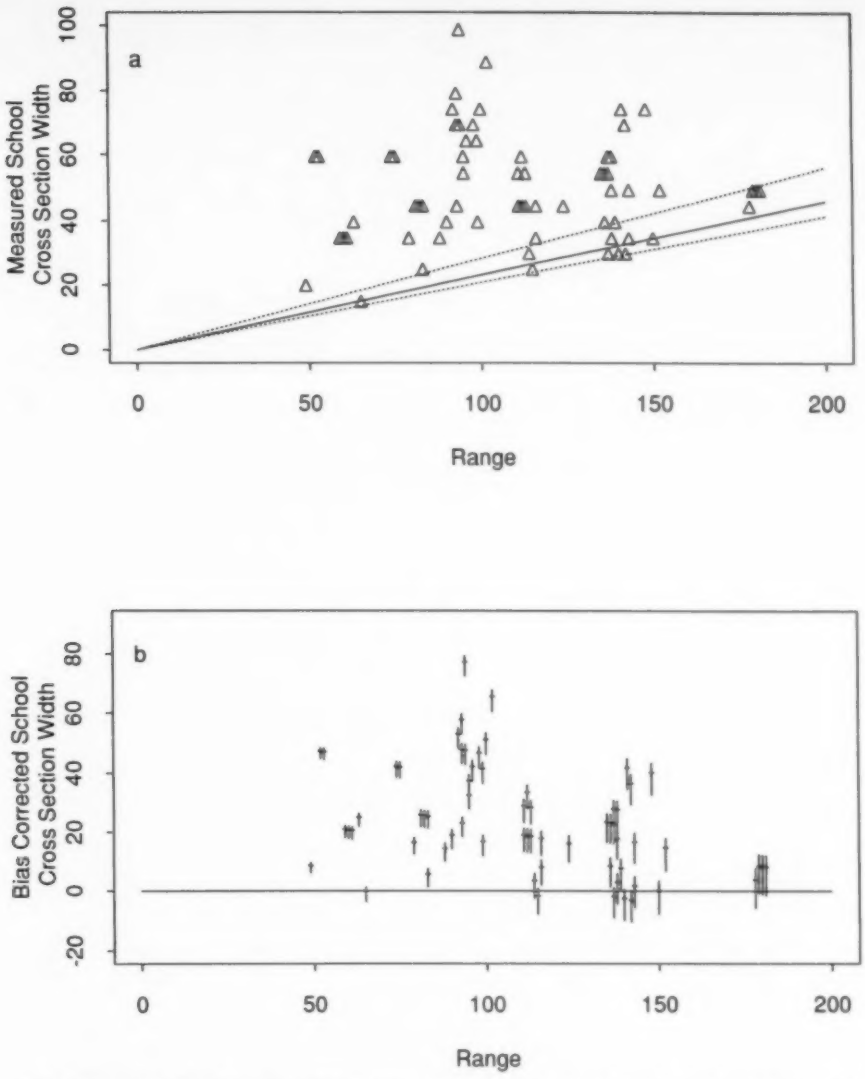


FIG. 9. Measured school cross section width (C_s) and the bias-corrected school cross section width (C_s^*) (m) versus the range (m) below the transducer. (a) Solid line corresponds to the beam width with the beam angle given by the estimated critical angle, $\hat{\theta}_c$, and its 95% confidence bounds shown by the broken lines. The vertical distance between the measured cross section width and the beam width at that range represents the corrected cross section width. (b) Bias-corrected cross section widths (horizontal lines) and the 95% confidence limits for each cross section width (vertical lines).

suggested by Smith (1970) and reviewed by MacLennan and Simmonds (1992) for sonar mapping of shallow schools. The bias correction of Smith is identical to (6) with the following restrictions: (i) Only a single R value (the average of the maximum and minimum ranges observed for the school) was used for each school. (ii) The beam angle was assumed to be θ_0 and no attempt was made to determine an effective beam angle, θ_c. (iii) Since Smith was interested mainly in larger schools, no correction for the school boundary due to the discrete measurement process was made, i.e., the correction made to (2a) to obtain (2b) was not used. Thus, our work extends Smith's method in each of these three areas. As a measure of the importance of these extensions, we note that if we used θ_0 in (6) rather than θ_c, then because

$$\frac{\sin \theta_0}{\sin \theta_c} = 0.62 ,$$

the estimate of the bias would be only 60% as large as the one we obtained.

Our selection criteria for single fish and school traces follow common practice. Commercial dual- and split-beam echo processing systems include single fish pulse recognition (Ehrenberg 1981; Knudsen 1990). In some cases, single fish traces are also identified. The recognition and processing of echoes from

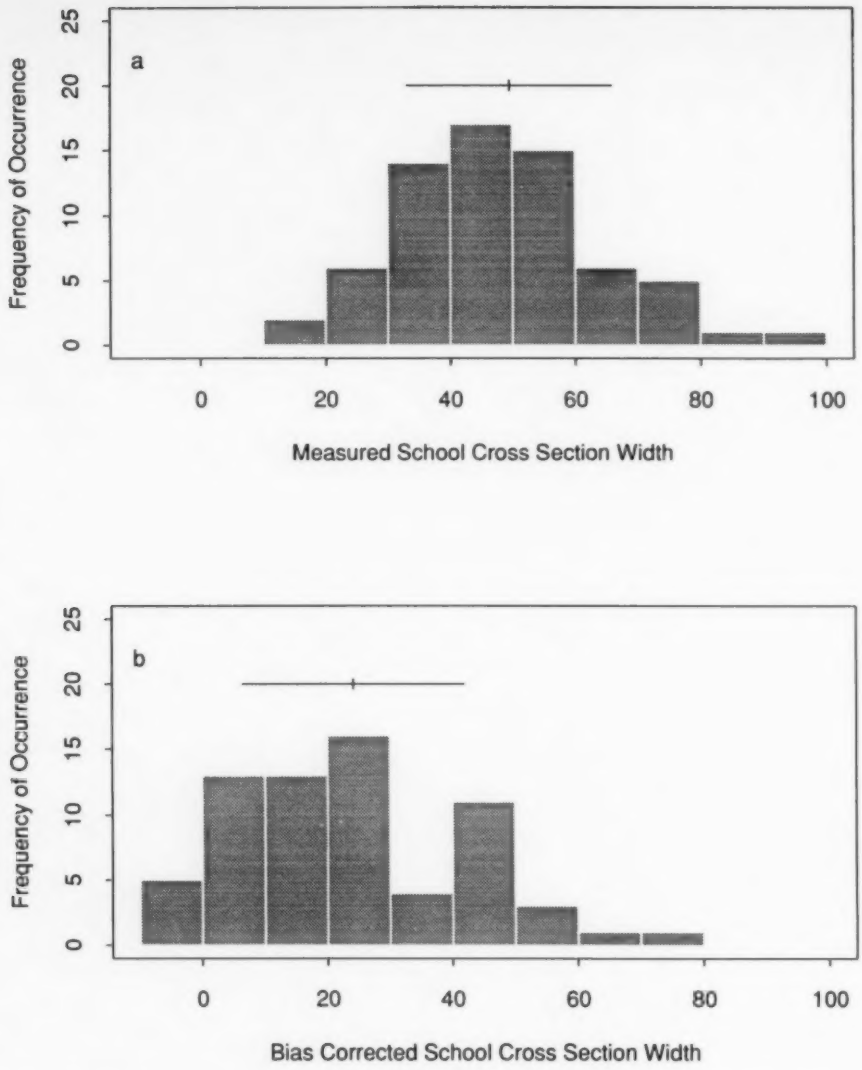


FIG. 10. Histograms of the (a) observed and (b) bias-corrected school cross section widths (m). The horizontal line above each histogram spans 1 SD on either side of the mean, indicated by the small vertical line. The five negative-value observations of the corrected cross section widths corespond to the misclassified school cross section widths shown in Fig. 5.

individual schools is less common. Kieser and Langford (1991) and Reid and Simmonds (1993) used image processing techniques to define and analyze echoes from individual fish schools. Diner et al. (1990) described a commercial echo processing system with this capability but gave little detail on how schools are defined. Our method was not developed for such broad application. It was intended for this study only. We did not attempt to define specific criteria for optimizing estimation of the beam width; rather, we sought to reproduce the normal criteria for visual examination of an echogram. Similarly, the school selection criteria were simple and were not intended to be universal. Other school section criteria, for example, the inclusion of information from adjacent rows of $\boldsymbol{W}$, might be necessary to accomplish broader goals, such as the determination of mean school density.

Our success in correcting the bias was due to two of the schooling characteristics of these rockfish. First, their tendency to form schools with extremely narrow cross section widths enabled us to use both C_f and C_s to estimate θ_c. Second, the fish were evenly distributed within the school, i.e., they did not tend to cluster together and thus to appear as a single, larger target. For example, if fish tend to associate within a school as closely separated pairs (closer together than the resolution of the acoustic system), an acoustic image of a single fish with a much larger target strength would result. Schools composed of such pairs would only begin to have a straight lower boundary when

the threshold of V_t was much higher than that observed for individual fish (Fig. 6).

Our work demonstrates the need for caution when attempting to extract additional information from acoustic data. Undoubtedly, more problems will arise when examining other new aspects of the acoustic signal. The solution to these problems can help fisheries management by supplying answers of greater precision and accuracy, previously unobtainable with less sophisticated acoustic data processing.

Acknowledgements

The authors wish to thank Rob Kronlund for his careful reading of our manuscript and for his suggestions for its improvement. Martial Thiebaux and an anonymous referee provided additional suggestions that helped improve our presentation.

References

BURCZYNSKI, J. 1982. Introduction to the use of sonar systems for estimating fish biomass. FAO Fish. Tech. Pap. 93 p.

CLARK, M.R., AND D.M. TRACEY. 1991. Trawl survey of orange roughy on the Challenger Plateau, July 1990. N.Z. Fish. Tech. Rep. No. 26: 20 p.

CLARK, M.R., AND D.M. TRACEY. 1992. Trawl survey of orange roughy in southern New Zealand waters, June–July 1991. N.Z. Fish. Tech. Rep. No. 32: 27 p.

CLAY, C.S. 1983. Deconvolution of the fish scattering PDF from the echo PDF for a single transducer sonar. J. Acoust. Soc. Am. 73: 1989–1994.

CLAY, C.S., AND B.G. HEIST. 1984. Acoustic scattering by fish — acoustic models and a two-parameter fit. J. Acoust. Soc. Am. 75: 1077–1083.

CLAY, C.S., AND H. MEDWIN. 1977. Acoustical oceanography: principles and applications. John Wiley & Sons Ltd., London, England. 544 p.

CRAIG, R.E., AND S.T. FORBES. 1969. Design of a sonar for fish counting. Fiskeridir. Skr. Ser. Havunders. 15: 210–219.

CRITTENDEN, R.N., AND G.L. THOMAS. 1992. A comparison of four duration-in-beam estimators for the sine of the half-angle of the effective conical volume sampled. Fish. Res. 14: 197–208.

CRITTENDEN, R.N., G.L. THOMAS, D.A. MARINO, AND R.E. THORNE. 1988. A weighted duration-in-beam estimator for the volume sampled by a quantitative echosounder. Can. J. Fish. Aquat. Sci. 45: 1249–1256.

DINER, N., A. WEILL, J.Y. COAIL, AND J.M. COUDEVILLE. 1990. INES:MOVIES: a new acoustic data acquisition and processing system, p. 939–942. *In* Congres Francais d'Acoustic 1990. Colloque de Physique. Colloque C2, supplement au n° 2, Tome 51, Fevrier 1990.

DUDA, R.O., AND P.E. HART. 1973. Pattern classification and scene analysis. John Wiley & Sons, Inc., New York, N.Y. 482 p.

EFRON, B. 1982. The jackknife, the bootstrap and other resampling plans. SIAM-CBMS 38: 1–92.

EHRENBERG, J.E. 1981. Analysis of split beam backscattering cross section estimation and single echo isolation. Rep. Appl. Phys. Lab. Univ. Wash. APL-UW 8108: 24 p.

FORBES, S.T., AND O. NAKKEN. 1971. Manual of methods for fisheries resources survey and appraisal. Part 2. The use of acoustic instruments for fish detection and abundance estimation. FAO Man. Fish. Sci. 5: 138 p.

KIESER, R., AND G. LANGFORD. 1991. An application of spatial analysis to fisheries acoustics, p. 335–339. *In* H. Schrier, S. Brown, P.O. Reilly, and P.J. Meehan [ed.] GIS'91: applications in a changing world. Minster of Supply and Services Canada, Cat. No. 29-19/153E.

KIESER, R., T.J. MULLIGAN, M.J. WILLIAMSON, AND M.O. NELSON. 1987. Intercalibration of two echo integration systems based on acoustic backscattering measurements. Can. J. Fish. Aquat. Sci. 44: 562–572.

KNUDSEN, H.P. 1990. The Bergin echo integrator: an introduction. J. Cons. Int. Explor. Mer 47: 167–174.

LEAMAN, B.M., R. KIESER, P. WITHLER, AND R.D. STANLEY. 1990. *W.E. Ricker* hydroacoustic cruise to study rockfish behaviour off northern Vancouver Island, March 14–23, 1990. Can. MS Rep. Fish. Aquat. Sci. 2091: 63 p.

MACLENNAN, D.N., AND E.J. SIMMONDS. 1982. Fisheries acoustics. Chapman & Hall, London, England. 325 p.

MATTHEWS, K.R., J.R. CANDY, L.J. RICHARDS, R. KIESER, AND C.M. HAND. 1989. Exploratory fishing and hydroacoustic observations of rockfish off Brooks Peninsula, Vancouver Island, from the *M/V Velma C* and *CSS John P. Tully*, March 13–23, 1989. Can. MS Rep. Fish. Aquat. Sci. 2029: 101 p.

MEGREY, B.A. 1991. Population dynamics and management of walleye pollock (*Theragra chalcogramma*) in the Gulf of Alaska, 1976–1986. Fish. Res. 11: 321–354.

MITSON, R.B. 1983. Fisheries sonar. Fishing News Books Ltd., Farnham, Surrey, England. 287 p.

NUNNALLEE, E.P. JR. 1980. Application of an empirically scaled digital echo integrator for assessment of juvenile salmon (*Oncorhynchus nerka* Walbaum) populations. Ph.D. dissertation, University of Washington, Seattle, Wash. 151 p.

REID, D.G., AND E.J. SIMMONDS. 1993. Image analysis techniques for the study of fish school structure from acoustic survey data. Can. J. Fish. Aquat. Sci. 50: 886–893.

RICHARDS, L.J., R. KIESER, T.J. MULLIGAN, AND J.R. CANDY. 1991. Differentiation of fish assemblages based on echo integration data. Can. J. Fish. Aquat. Sci. 48: 1264–1272.

SMITH, P.E. 1970. The horizontal dimensions and abundance of fish schools in the upper mixed layer as measured by sonar, p. 563–600. *In* G.B. Farquhar. [ed.] Proceedings of an International Symposium on Biological Sound Scattering in the Ocean. Maury Center for Ocean Science, Department of the Navy, Washington, D.C. 629 p.

SULLIVAN, P.J. 1991. Stock abundance estimation using depth-dependent trends and spatially correlated variation. Can. J. Fish. Aquat. Sci. 48: 1691–1703.

CORRECTIONS TO RECENT PUBLICATIONS/ CORRECTIONS SUR RECENTES PUBLICATIONS

VOLUME 50, No. 1 — article by R.W. Gensemer, R.E.H. Smith, H.C. Duthie, and S.L. Schiff: *pH tolerance and metal toxicity in populations of the planktonic diatom* Asterionella*: influences of synthetic and natural dissolved organic carbon.*

Pages 125–127: In Fig. 1–6, all error bars represent ±1 SE about each mean growth rate, not ±2 SE as stated in the original figure legends.

VOLUME 50, No. 4 — article by D.P. Swain and E.J. Wade: *Density-dependent geographic distribution of Atlantic cod* (Gadus morhua) *in the southern Gulf of St. Lawrence.*

Page 730: In Fig. 5, right panels (ages 6, 7, and 8+), the abundance scale in millions (x-axis) should read 50, 100, and 150, not 100, 200, and 300 as in the published figure.

ANNOUNCEMENTS / AVIS

First Announcement

Call for Papers

Symposium on the Biology and Ecology of Northwest Atlantic Cod

24–28 October 1994
Radisson Plaza Hotel
St. John's, Newfoundland, Canada

Northern Cod Science Program (NCSP)
Ocean Production Enhancement Network (OPEN)
Memorial University of Newfoundland (MUN)

Objectives and Scope

This Symposium will review recent research on Atlantic cod undertaken by the Northern Cod Science Program, the Ocean Production Enhancement Network, and Memorial University of Newfoundland. Researchers not affiliated with these programs are also encouraged to participate. The goals of the Symposium will be to summarize current knowledge on cod biology and ecology with particular emphasis on the Northwest Atlantic, a forum for application of recent research results to current fisheries problems, and recommendations for research directions. The Symposium will include formal oral presentations, posters, and workshop discussions on relevant topics.

Call for Papers

Persons conducting cod-related research are invited to submit titles for paper or poster presentations no later than **1 December 1993**.

Presentations are encouraged on biological (e.g., phenology, physiology, genetics, growth, survival), ecological (e.g., distribution, abundance, behaviour, predator/prey interactions), and physical (e.g., transport, models) processes and on new research methods and technologies.

Titles, authorship, affiliation, and paper/poster preference should be sent to Symposium on Biology and Ecology of Northwest Atlantic Cod, Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada. Telephone: (709) 772-2051; FAX: (709) 772-6100.

Abstracts of papers and posters will be requested in early 1994.

Papers and posters will be eligible for primary publication in a supplement of the *Canadian Journal of Fisheries and Aquatic Sciences* subject to the normal peer review process. Details on publication procedures will be distributed in early 1994.

Première annonce

Demande de communications

Symposium sur la biologie et l'écologie de la morue de l'Atlantique nord-ouest

Du 24 au 28 octobre 1994
Hôtel Radisson Plaza
St. John's, Terre-Neuve, Canada

Programme de recherche scientifique sur la morue du nord
Réseau de mise en valeur des ressources maritimes
Université Memorial de Terre-Neuve

Portée et objectifs

Au cours de ce symposium, on examinera les recherches sur la morue fraîche récemment entreprises par le Programme de recherche scientifique sur la morue du nord, le Réseau de mise en valeur des ressources maritimes et l'Université Memorial de Terre-Neuve. Les chercheurs non affiliés à ces organisations sont également invités à participer. Les objectifs du symposium consistent à faire le bilan des connaissances actuelles sur la biologie et l'écologie de la morue en mettant l'accent sur l'Atlantique nord-ouest, d'appliquer les résultats obtenus récemment aux problèmes halieutiques actuels et de formuler des recommandations pour orienter les recherches à venir. Le symposium comprendra des exposés oraux, des séances d'affichage et des ateliers sur des sujets d'intérêt.

Demande de communications

Les personnes qui mènent des recherches sur la morue sont invitées à soumettre, d'ici **le 1 décembre 1993**, des titres d'exposés ou de présentations.

On encourage les intéressés à soumettre des présentations sur les processus biologiques (phénologie, physiologie, génétique, croissance, survie), écologiques (distribution, abondance, comportement, interactions prédateur–proie) et physiques (transport, modèles), de même que sur de nouvelles méthodes et techniques de recherche.

Les titres de présentations, accompagnés du nom des auteurs ainsi que de celui des organisations auxquelles ceux-ci sont affiliés, doivent être envoyés à l'adresse suivante : Symposium sur la biologie et l'écologie de la morue de l'Atlantique nord-ouest, Ministère des Pêches et des Océans, Sciences, C.P. 5667, St. John's, NF A1C 5X1, Canada. Téléphone : (709) 772-2051; télécopieur : (709) 772-6100.

On demandera aux candidats d'envoyer un résumé de leur exposé ou de leur présentation pour affichage au début de 1994.

Ces exposés et présentations pourront faire l'objet d'une première publication dans un supplément du *Journal canadien des sciences halieutiques et aquatiques*, sous réserve de l'examen d'usage par les pairs. La marche à suivre pour la publication sera distribuée au début de 1994.

AMPHIPACIFICA

AMPHIPACIFICA is a new international journal of invertebrate systematics, aimed primarily at the publication of monographic treatments that are too large or bulky (50–100 printed pages, including plates) for acceptance by regular taxonomic journals such as the *Journal of Crustacean Biology* or the *Canadian Journal of Zoology*. Initially, the contents will feature monographic studies on crustaceans of the faunistically rich and geologically ancient North Pacific coastal marine region. The scope of this new journal extends to other arthropods, mollusks, annelids, and to other regional invertebrate taxa, both aquatic and terrestrial, including parasites, and to aspects of vertebrate animals that may involve invertebrates.

The journal is to appear quarterly, or approximately so, with a run of 300–400 copies per issue, each of 200–225 pages, and a Volume (yearly) total of approximately 1100 pages. Printed page size is 8.5 × 11 inches (22 × 27.5 cm). Paper quality will accommodate linecuts and halftones at 400–600 d.p.i. and a limited number of colour plates at author cost. Manuscripts are to be submitted in "camera-ready" computerized format (IBM- or Mac-compatible diskettes) that have previously been refereed and text edited at the author's instigation. Suitability of manuscripts, based on content and adherence to submission regulations, will be decided by the Advisory Board of the new journal.

The cost of printing and mailing of each issue will be defrayed by institutional and individual subscriptions to the journal of $50.00 Canadian funds ($40.00 U.S.) per annum and by page charges to the authors of $15.00 per printed page (including plates). For further information please address all correspondence to Dr. E.L. Bousfield, Managing Editor, AMPHIPACIFICA, Royal British Columbia Museum, 675 Belleville St., Victoria, BC V8V 1X4, Canada. Telephone: (604) 380-3787; FAX: (604) 356-8197.

AMPHIPACIFICA

AMPHIPACIFICA est une nouvelle revue mondiale de systématique des invertébrés dont l'objectif principal est de publier des études monographiques trop longues (50 à 100 pages imprimées, planches comprises) pour être acceptées par les revues de taxonomie ordinaires comme le *Journal of Crustacean Biology* ou la *Revue canadienne de zoologie*. Dans un premier temps, la revue présentera des études monographiques sur les crustacés de la côte du Pacifique Nord, une région riche en faune et géologiquement ancienne. Elle se penchera également sur d'autres arthropodes, mollusques et annélides, ainsi que sur des taxums d'invertébrés aquatiques et terrestres et sur des vertébrés dont certains aspects sont liés aux invertébrés.

La revue paraîtra tous les trois mois, ou à peu près, et aura un tirage de 300 à 400 exemplaires. Chaque numéro contiendra 200 à 225 pages et le volume total (annuel) sera d'environ 1100 pages. Le format de la page imprimée sera de 8,5 × 11 pouces (22 × 27,5 cm). La qualité du papier permettra l'utilisation de clichés de trait et de demi-teintes avec une résolution de 400 à 600 points au pouce et l'impression, aux frais de l'auteur, d'un nombre limité de planches de couleur. Les manuscripts devront être envoyés sous forme de prêts-à-photographier sur support électronique (disquettes compatibles avec IBM ou Mac). Il incombe à l'auteur de soumettre son texte à l'examen d'un comité de lecture et de le mettre en forme avant de l'envoyer. Le conseil consultatif de la revue approuvera ou rejettera les manuscrits en fonction de leur contenu et du respect des critères de présentation.

Les coûts d'impression et d'expédition de la revue seront couverts par les abonnements, qui seront de 50 $ (40 $ U.S.) par an, ainsi que par les frais de publication de 15 $ la page imprimée (y compris les planches) devant être assumés par les auteurs. Pour de plus amples renseignements, n'hésitez pas à communiquer avec : E.L. Bousfield, Rédacteur administratif, AMPHIPACIFICA, Royal British Columbia Museum, 675, Belleville Street, Victoria, BC V8V 1X4, Canada. Téléphone : (604) 380-3787; télécopieur : (604) 356-8197.

THE INFORMATION ADVANTAGE

The most up-to-date scientific, technical and medical information in the world is at your fingertips. Without leaving your office you can tap into the largest scientific and technical library in North America, as well as a global network of databases.

Speak to one of our information specialists or gain on-line access yourself. Either way you'll have the precise information you need, within a few hours if required.

Stay on top of the latest developments in your field with NRC's Canada Institute for Scientific and Technical Information (CISTI). It's an efficient and cost-effective way to give your company the competitive edge.

Call **1-800-668-1222** (or fax 613-952-9112) for a brochure about CISTI, and get the information advantage.

L'AVANTAGE DE L'INFORMATION

L'information scientifique, technique et médicale la plus récente du monde est au bout de vos doigts. Sans même quitter votre bureau, vous pouvez avoir accès à l'Institut canadien de l'information scientifique et technique (ICIST) du CNRC, la plus vaste bibliothèque de science et de technologie d'Amérique du Nord ainsi qu'à un réseau international de bases de données.

Que ce soit par accès direct ou par l'entremise de nos spécialistes, vous obtiendrez l'information précise dont vous avez besoin en quelques heures s'il le faut.

Demeurez au fait des découvertes dans votre domaine grâce aux moyens efficaces et économiques que vous offre l'ICIST.

Composez le **1-800-668-1222** (ou télécopiez-nous au 613-952-8112) pour obtenir une brochure sur les services de l'ICIST et accordez-vous l'avantage de l'information.

NRC · CNRC

National Research Council Canada | Conseil national de recherches Canada

Canada

Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) and their North American fisheries

In the Canadian Bulletin of Fisheries and Aquatic Sciences No. 227

Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) off the Atlantic and Pacific coasts of North America share many biological characteristics. The conventional separation of the halibut into separate species is not uniformly accepted. Reproduction and early life history are nearly identical, although the extensive drift of Pacific halibut eggs and larvae and countermigration as juveniles has not been demonstrated for the Atlantic halibut. Adult halibut undergo migration from winter spawning grounds along the upper continental slope to summer feeding grounds on the continental shelf. The two halibuts show the same pattern of age and growth. They exhibit different trophic selectivity, as small Atlantic halibut consume a higher proportion of invertebrates as small fish and large Atlantic halibut consume a higher proportion of fish, compared to Pacific halibut. Halibut management in the Atlantic and Pacific varies dramatically.

84 pages

AUTHORS
R.J. Trumble, J.D. Neilson, W.R. Bowering, and D.A. McCaughran

To order copies of this publication, complete the order form below.

Please send me ___copies of the Canadian Bulletin of Fisheries and Aquatic Sciences No. 227. (FCB 267)

Canada $27.00 (CAN) ea.* _____

Other countries $27.00 (US) ea.* _____

Make cheque or money order payable to "Receiver General for Canada, credit NRC" and send to

Subscriptions Office, Building M-55
Research Journals
National Research Council Canada
Ottawa, ON K1A 0R6, Canada

*Canadian customers please add 7% GST.
In Quebec please add a further 8% QST to the total price including GST.

PLEASE PRINT

Visa ☐ MasterCard ☐ Expiry date month ____ year ____

Account No. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Name _____

Signature _____

Address _____

City _____ Province/State _____

Postal code/Zip code _____ Country _____

Telephone No. _____ Fax No. _____

NRC · CNRC

Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) and their North American fisheries

Dans le Bulletin canadien des sciences halieutiques et aquatiques n° 227

Le flétan de l'Atlantique (*Hippoglossus hippoglossus*) et le flétan du Pacifique (*H. stenolepis*) qui vivent au large des côtes de l'Atlantique et du Pacifique de l'Amérique du Nord ont de nombreuses caractéristiques biologiques en commun. La séparation traditionnelle du flétan en espèces distinctes n'est pas uniformément acceptée. La reproduction et les premiers stades de vie sont presque identiques, bien que la dérive sur une grande distance des oeufs et des larves de flétan du Pacifique et la contre-migration des juvéniles n'aient pas été mises en évidence dans le cas du flétan de l'Atlantique. Les flétans adultes migrant depuis les aires de frai d'hiver situées le long de la partie supérieure du talus continental pour se rendre dans les aires d'alimentation d'été situées sur le plateau continental. Les deux espèces de flétan montrent les caractéristiques semblables du point de vue de l'âge et de la croissance. Elles présentent une sélectivité trophique différent du fait que,comparativement au flétan du Pacifique, le flétan de l'Atlantique de petite taille consomme une proportion plus élevée d'invertébrés et le flétan de l'Atlantique de grande taille consomme une proportion plus élevée de poissons. La gestion du flétan dans l'Atlantique et dans le Pacifique est totalement différente.

AUTEURS
R.J. Trumble, J.D. Neilson, W.R. Bowering, and D.A. McCaughran

Pour commander des copies de ce numéro, veuillez remplir le bon de commande ci-dessous.

Veuillez me faire parvenir_____exemplaire(s) de la publication spéciale canadienne des sciences halieutiques et aquatiques n° 227. (Code Financier 267)

Canada 27,00 $CAN chac.* _____

Autres pays 27,00 $US chac.* _____

Libellez le chèque ou mandat payable au «Receveur général du Canada, au crédit du CNRC» et envoyez à l'adresse suivante :

Bureau d'abonnements, Édifice M-55
Revues scientifiques
Conseil national de recherches du Canada
Ottawa, ON K1A 0R6, Canada

*Clients canadiens veuillez ajouter 7% TPS.
Au Québec veuillez ajoutez un 8% de plus pour la TVQ au montant total incluant la TPS.

LETTRES MOULÉES

Visa ☐ MasterCard ☐ Date d'expiration mois ____ année ____

N° de compte ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Nom _____

Signature _____

Adresse _____

Ville _____ Province _____

Code postal _____ Pays _____

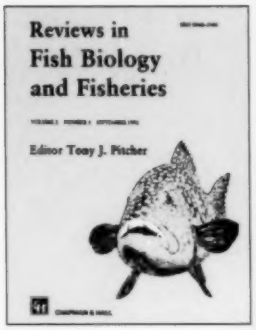
N° de téléphone _____ N° de télécopieur _____

CNRC · NRC

CHAPMAN & HALL

Reviews in Fish Biology and Fisheries

Editor: **Tony Pitcher,** University of British Columbia, Vancouver, Canada



Reviews in Fish Biology and Fisheries is a quarterly international journal which publishes review articles on varied aspects of fish and fisheries biology. Reviews are accepted in any field of fish biology where the emphasis is on the whole organism. The subjects covered include physiology, evolutionary biology, taxonomy, zoogeography, behaviour, ecology and exploitation. The reviews provide an up-to-date synopsis for research workers in the chosen field with an outline of the next problems which should be tackled. They also provide the non-specialist fish biologist with an awareness and understanding of the field.

In addition to review articles, details of notable papers given at recent conferences, book reviews and notices of forthcoming conferences are included.

The Journal caters for all those with an interest in fish biology and fisheries including those from universities and research institutes, fishing industries, local, regional and government institutions and international organizations.

A Selection of Papers

Neural mechanisms underlying escape behaviour in fishes. **B L Roberts (The Netherlands).** Fisheries for small pelagic species: an empirical approach to management targets. **K Patterson (UK).** Gill surface area of water-breathing freshwater fish. **M Palzenberger** and **H Pohla (Austria).** The impact of introduced brown and rainbow trout on native fish: the case of Australia. **T A Crowl, C R Townsend** and **A R McIntosh (USA and New Zealand).** Parasites as biological indicators of the population biology, migrations, diet and phylogenetics of fish. **H H Williams, K MacKenzie** and **A M McCarthy (UK).** Human-induced changes in the composition of fish communities in the African Great Lakes. **J F Craig (UK).**

The papers published in **Reviews in Fish Biology and Fisheries** are included in *Cambridge Scientific Abstracts* and *Current Awareness in Biological Sciences.*

Subscription Information

ISSN: 0960-3166
Published quarterly
Volume 3 will be published in 1993

Individual Rates	
European Community: £99	European Community: £32
USA/Canada: $185	USA/Canada: $59
Rest of World: £110	Rest of World: £32

For a free sample copy, or to subscribe, please contact:
Journals Promotion Dept., Chapman & Hall,
2-6 Boundary Row, London SE1 8HN, UK
Tel: +44 (71) 865 0066 Fax: +44 (71) 522 9623 email: journal@chall.mhs.compuserve.com
or
Journals Promotion Dept., Chapman & Hall,
29 West 35th Street, New York, NY 10001-2299, USA
Tel: +1 (212) 244 3336 Fax: +1 (212) 563 2269 email: 71201.1651.@compuserve.com

CHAPMAN & HALL

1993

Annual Subscription Rates / Abonnements annuels*†‡

Publication	Volume	Code	Canada ($CAN) Institutional/ Collectif (CI)	Canada ($CAN) Personal/ Personnel (CP)	Foreign / Étranger ($US) Institutional/ Collectif (FI)	Foreign / Étranger ($US) Personal/ Personnel (FP)	Foreign / Étranger ($US) Air mail / Par avion Add / ajouter
Monthly / Mensuelle							
Biochemistry and Cell Biology / Biochimie et biologie cellulaire	71	44	237	88	237	90	75
Canadian Journal of Botany / Revue canadienne de botanique	71	45	343	136	343	146	150
Canadian Journal of Chemistry / Revue canadienne de chimie	71	46	445	140	445	149	150
Canadian Journal of Earth Sciences / Revue canadienne des sciences de la Terre	30	47	335	108	335	113	150
Canadian Journal of Fisheries and Aquatic Sciences / Journal canadien des sciences halieutiques et aquatiques	50	261	305	117	350	129	150
Canadian Journal of Forest Research / Revue canadienne de recherche forestière	23	54	297	98	297	102	75
Canadian Journal of Microbiology / Revue canadienne de microbiologie	39	48	241	95	241	97	75
Canadian Journal of Physics / Revue canadienne de physique	71	49	285	94	285	96	75
Canadian Journal of Physiology and Pharmacology / Revue canadienne de physiologie et pharmacologie	71	50	327	107	327	112	75
Canadian Journal of Zoology / Revue canadienne de zoologie	71	51	368	141	368	151	150
Bimonthly / Bimestrielle							
Canadian Journal of Civil Engineering / Revue canadienne de génie civil	20	52	238	77	238	79	75
Genome / Génome	36	70	219	90	219	92	75
Canadian Geotechnical Journal / Revue canadienne de géotechnique	30	53	190	72	190	76	50
Quarterly / Trimestrielle							
Environmental Reviews/ Dossiers environnement	1	244	160	55	160	60	25

*Price of regular single issues of all journals: institutional, $39.00; personal, $19.00. / Prix des numéros réguliers de toutes les revues : collectif, 39,00 $; personnel, 19,00 $.

†Rates include second-class postage. Canadian customers please include additional 7% GST (GST No. R121491807). Quebec customers please add 8% QST to the total price including GST (QST No. 1006178088). / Affranchissement de deuxième classe compris. Clients canadiens veuillez ajouter 7% pour la TPS (N° TPS R121491807). Clients du Québec veuillez ajouter 8% TPQ au montant total incluant TPS (N° TPQ 1006178088).

‡Special rates apply for members of Canadian scientific and engineering societies if they subscribe through their respective societies. / Les tarifs spéciaux sont offerts aux membres des sociétés scientifiques et des sociétés de génie au Canada, s'ils s'abonnent par l'intermédiaire de leur société respective.

Subscriptions, renewals, requests for single or back numbers, and all remittances should be sent to Subscription Office, Research Journals, National Research Council of Canada, Ottawa, ON, Canada K1A 0R6. Remittances should be made payable to the Receiver General for Canada, credit National Research Council of Canada. Enquiries: Helen Goulet 613-993-9084. Fax No. 613-952-7656.

Subscriptions are entered for the calendar year. There are no refunds on cancelled subscriptions once delivery has begun.

Change of address - Send change of address information (complete old address, customer code, and new address) to Subscription Office, Research Journals, National Research Council of Canada, Ottawa, ON, Canada K1A 0R6.

Claims for missing numbers should be made within four months (North America) or six months (outside North America) following the date of publication. The publisher expects to supply missing numbers only when losses have been sustained in transit and when the reserve stock will permit.

Abonnements - Pour toutes les questions d'abonnements, paiements, demandes de vieux numéros de la revue, etc., on doit s'adresser au Bureau d'abonnements, Revues scientifiques, Conseil national de recherches du Canada, Ottawa, ON, Canada K1A 0R6. Les chèques et mandats seront formulés à l'ordre du Receveur général du Canada, au crédit du Conseil national de recherches du Canada. Renseignements : Helen Goulet 613-993-9084. N° de télécopieur 613-952-7656.

La durée des abonnements correspond à l'année civile. Les abonnements annulés ne pourront être remboursés une fois que le premier numéro sera envoyé.

Changement d'adresse - Faire parvenir tous renseignements (ancienne adresse, code du client et nouvelle adresse) au Bureau d'abonnements, Revues scientifiques, Conseil national de recherches du Canada, Ottawa, ON, Canada K1A 0R6.

Les réclamations de numéros manquants doivent être adressées dans les quatre mois (en Amérique du Nord) ou les six mois (à l'étranger) suivant la date de publication. L'éditeur s'engage à remplacer les numéros manquants seulement lorsque les pertes ont été subies en transit et lorsque ses réserves le permettent.

National Research Council Canada
Ottawa, Canada K1A 0R6

Conseil national de recherches Canada
Ottawa, Canada K1A 0R6

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 50, No. 8, August 1993 **Volume 50, nº 8, août 1993**

Myers, R.A., and N.G. Cadigan. Density-dependent juvenile mortality in marine demersal fish 1576–1590
Myers, R.A., and N.G. Cadigan. Is juvenile natural mortality in marine demersal fish variable? 1591–1598
Myers, R.A., K.F. Drinkwater, N.J. Barrowman, and J.W. Baird. Salinity and recruitment of Atlantic cod (*Gadus morhua*) in the Newfoundland region 1599–1609
Gottgens, J.F., and T.L. Crisman. Quantitative impacts of lake-level stabilization on material transfer between water and sediment in Newnans Lake, Florida 1610–1616
Rijnsdorp, A.D. Relationships between juvenile growth and the onset of sexual maturity of female North Sea plaice, *Pleuronectes platessa* 1617–1631
Nicieza, A.G., and F. Braña. Relationships among smolt size, marine growth, and sea age at maturity of Atlantic salmon (*Salmo salar*) in northern Spain 1632–1640
Ward, R.D., and N.G. Elliott. Heterozygosity and morphological variability in the orange roughy, *Hoplostethus atlanticus* (Teleostei: Trachichthyidae) 1641–1649
Walters, C.J. Spatial model for the population dynamics and exploitation of the Western Australian rock lobster, *Panulirus cygnus* 1650–1662
Lancaster, J., and A.G. Hildrew. Characterizing in-stream flow refugia 1663–1675
Vijayan, M.M., A.G. Maule, C.B. Schreck, and T.W. Moon. Hormonal control of hepatic glycogen metabolism in food-deprived, continuously swimming coho salmon (*Oncorhynchus kisutch*) 1676–1682
Miller, P.A., R.P. Lanno, M.E. McMaster, and D.G. Dixon. Relative contributions of dietary and waterborne copper to tissue copper burdens and waterborne-copper tolerance in rainbow trout (*Oncorhynchus mykiss*) 1683–1689
Rice, J.C. Forecasting abundance from habitat measures using nonparametric density estimation methods 1690–1698
Kieffer, J.D., and P.W. Colgan. Foraging flexibility in pumpkinseed (*Lepomis gibbosus*): influence of habitat structure and prey type 1699–1705
Novales Flamarique, I., and C.W. Hawryshyn. Spectral characteristics of salmonid migratory routes from southern Vancouver Island (British Columbia) 1706–1716
Hontela, A., J.B. Rasmussen, K. Lederis, H.V. Tra, and G. Chevalier. Elevated levels of arginine vasotocin in the brain of brook trout (*Salvelinus fontinalis*) from acid lakes: a field test of a potential biomarker for acid stress 1717–1727
Nürnberg, G.K., and P.J. Dillon. Iron budgets in temperate lakes 1728–1737
Devlin, R.H. Sequence of sockeye salmon type 1 and 2 growth hormone genes and the relationship of rainbow trout with Atlantic and Pacific salmon 1738–1748
Overholtz, W.J. Harvesting strategies and fishing mortality reference point comparisons for the Northwest Atlantic stock of Atlantic mackerel (*Scomber scombrus*) 1749–1756
Carpenter, S.R., R.C. Lanthrop, and A. Muñoz-del-Rio. Comparison of dynamic models for edible phytoplankton 1757–1767
Bowen, W.D., J.W. Lawson, and B. Beck. Seasonal and geographic variation in the species composition and size of prey consumed by grey seals (*Halichoerus grypus*) on the Scotian Shelf 1768–1778
Malley, D.F., P.C. Williams, M.P. Stainton, and B.W. Hauser. Application of near-infrared reflectance spectroscopy in the measurement of carbon, nitrogen, and phosphorus in seston from oligotrophic lakes 1779–1785
Tang, M., and D. Boisclair. Influence of the size of enclosures on the swimming characteristics of juvenile brook trout (*Salvelinus fontinalis*) 1786–1793
Gobeil, C., and D. Cossa. Mercury in sediments and sediment pore water in the Laurentian Trough 1794–1800
Kieser, R., T.J. Mulligan, L.J. Richards, and B.M. Leaman. Bias correction of rockfish school cross section widths from digitized echo sounder data 1801–1811

Corrections to recent publications/Corrections sur récentes publications 1812
Announcements/Avis 1813–1814

CJFSDX50(8) 1575–1814 (1993)
ISSN 0706-652X

Canada

Typeset and printed in Canada by /
Composé et imprimé au Canada par
The Lowe-Martin Group: Runge Press Division

0706-652X(199308)50:8;1-4